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REVIEWS OF THE ENVIRONMENTAL EFFECTS OF POLLUTANTS:

XI. Chlorophenols

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REVIEWS OF THE ENVIRONMENTAL EFFECTS OF POLLUTANTS: XI. CHLOROPHENOLS

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FOREWORD

A vast amount of published material is accumulating as numerous research investigations are conducted to develop a data base on the adverse effects of environmental pollution. As this information is amassed, it becomes continually more critical to focus on pertinent, well-designed studies. Research data must be summarized and interpreted in order to adequately evaluate the potential hazards of these substances to ecosystems and ultimately to public health. The Reviews of the Environmental Effects of Pollutants (REEPs) series represents an extensive compilation of relevant research and forms an up-to-date compendium of the environmental effect data on selected pollutants.

Reviews of the Environmental Effects of Pollutants: XI. Chlorophenols includes information on chemical and physical properties; pertinent analytical techniques; transport processes to the environment and subsequent distribution and deposition; impact on microorganisms, plants, and wildlife; toxicologic data in experimental animals including metabolism, toxicity, mutagenicity, teratogenicity, and carcinogenicity; and an assessment of its health effects in man. The large volume of factual information presented in this document is summarized and interpreted in the final chapter, "Environmental Assessment," which presents an overall evaluation of the potential hazard resulting from present concentrations of chlorophenols in the environment. This final chapter represents a major contribution by Gary A. Van Gelder from the University of Missouri.

The REEPs are intended to serve various technical and administrative personnel within the Agency in the decision-making processes, i.e., in the development of criteria documents and environmental standards, and for other regulatory actions. The breadth of these documents makes them a useful resource for public health personnel, environmental specialists, and control officers. Upon request these documents will be made available to any interested individuals or firms, both in and out of the government. Depending on the supply, the document can be obtained directly by writing to:

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ABSTRACT

This study reviews the health and environmental effects of chlorophenols. It includes discussions of physical and chemical properties; analytical methods; biological aspects in microorganisms, plants, animals, and humans; environmental distribution and transformation; and environmental interactions and their consequences. All of the economically or environmentally important chlorophenol isomers are reviewed: 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetrachlorophenols, and pentachlorophenol. Approximately 400 references are cited.

2-Chlorophenol is used mainly as a starting material for further chlorination to 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol. Similarly, 2,4-dichlorophenol and 2,4,5-trichlorophenol are utilized principally as intermediates for the synthesis of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) respectively. 2,4,5-Trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and their salts are important germicides for the preservation of wood, glue, latex, leather, and textiles. Pentachlorophenol has been widely used as a fungicide for wood preservation.

The toxicity of the chlorophenol isomers to higher organisms increases with increased chlorine substitution. Pentachlorophenol is the most toxic of the isomers. Fish kills, extensive losses of wildlife, toxic effects in domestic animals, and human fatalities have resulted from exposure to pentachlorophenol. Available evidence indicates that moderate bioconcentration and biomagnification of pentachlorophenol may occur in aquatic organisms.

The primary source of human exposure to chlorophenols (except pentachlorophenol) is likely the degradation of chemically related phenoxyalkanoic herbicides such as 2,4-D and 2,4,5-T. Use of pentachlorophenol as a wood preservative is the major source of human exposure. Chlorination of drinking water or wastewater may result in the production of chlorophenols. Several isomers possess potent organoleptic properties. Pentachlorophenol is ubiquitous in the environment. Low, but detectable, levels have been found in river water, municipal water supplies, human foodstuffs, and in the blood, urine, and fat of nonoccupationally exposed humans. The exact sources of these residues have not been identified. Monitoring data on the other chlorophenol isomers are not available.

Chronic toxicity of chlorophenols to humans has not been documented. Available data indicate that chlorophenols do not possess tumorigenic, mutagenic, or teratogenic properties. Several isomers may be embryotoxic. 2-Chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol may promote tumors in mice. Available data fail to suggest a direct carcinogenic potential of any of the isomers reviewed.

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PART A

SUMMARY OF COMPARATIVE RELATIONSHIPS OF CHLOROPHENOLS

A.1 INTRODUCTION

Part A provides a comparative overview of the environmental effects of chlorophenols. Whenever possible, comparisons are made among the compounds 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetrachlorophenols, and pentachlorophenol. A lack of information indicates that valid comparisons based on the available data cannot be made. In the interest of clarity and readability, brief summary statements are occasionally used when individual compounds are discussed even though a lack of adequate data on the other compounds precludes a comparative statement. No attempt is made to summarize the data and conclusions completely. More detailed and complete summaries of the environmental and biological aspects of these compounds are found at the beginning of Parts B, C, and D of the report. In Part A, reference citations are used only when specific data are presented in tabular form. The reader is referred to individual sections and their bibliographies for complete reference information.

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A.2 PHYSICAL AND CHEMICAL PROPERTIES AND ANALYSIS

A.2.1 PHYSICAL PROPERTIES

All chlorophenols listed in Table A.2.1 are solids at room temperature except 2-chlorophenol, which is a liquid. They have pungent, medicinal odors which are more intense and persistent with 2-chlorophenol and 2,4-dichlorophenol. A wide variation in boiling points, which increase with increased chlorination, exists among chlorophenols. This variation allows convenient separation of the chemicals by fractional distillation. They can be steam distilled, but only 2-chlorophenol volatilizes from aqueous alkaline solutions. Chlorophenols behave as weak acids, and the acidity increases with increased chlorination, as shown by the dissociation constants. This behavior implies that ionization of higher chlorophenols in aqueous solutions occurs over a wider pH range (i.e., pentachlorophenol begins to dissociate at a pH of about 3.5, but 2-chlorophenol does not dissociate below pH 7). Dissociation of the chemicals influences their sorption on colloids and their toxicological properties. The toxicity of 2,4,6-trichlorophenol and pentachlorophenol to fungi is decreased as the degree of dissociation increases. Volatility and water solubility of chlorophenols decrease with increasing degree of chlorination. Although their solubility in water is low, they are readily soluble in many organic solvents. Partition coefficients in favor of the organic solvents facilitate isolation of the compounds for analysis.

A.2.2 MANUFACTURE

Two main processes are presently utilized in the manufacture of chlorophenols (Table A.2.2). The chemistry of these processes is well known and no patent currently exists. With the exception of 2,4,5-trichlorophenol, commercial synthesis of chlorophenols involves the direct chlorination of phenol to various extents until the desired product is obtained. The production of 2,4,5-trichlorophenol is accomplished by hydrolysis of 1,2,4,5-tetrachlorobenzene with sodium hydroxide.

A.2.3 USES

2-Chlorophenol is used mainly as a starting material for further chlorination to 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol (Table A.2.2). Similarly, 2,4-dichlorophenol and 2,4,5-trichlorophenol are utilized principally as intermediates for the synthesis of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) respectively. 2,4,5-Trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and their salts are important germicides for the preservation of wood, glue, latex, leather, and textiles. Pentachlorophenol has been widely used as a fungicide for wood preservation.

TABLE A.2.1. PHYSICAL PROPERTIES OF CHLOROPHENOLS

Property	2-Chlorophenol	2,4-Dichloro-phenol	2,4,5-Tri-chlorophenol	2,4,6-Tri-chlorophenol	2,3,4,6-Tetra-chlorophenol	2,3,4,5-Tetra-chlorophenol	2,3,5,6-Tetra-chlorophenol	Penta-chlorophenol
Empirical formula	C_6H_5ClO	$C_6H_4Cl_2O$	$C_6H_3Cl_3O$	$C_6H_3Cl_3O$	$C_6H_2Cl_4O$	$C_6H_2Cl_4O$	$C_6H_2Cl_4O$	C_6HCl_5O
Molecular weight	128.56	163.01	197.46	197.46	231.91	231.91	231.91	266.35
Melting point, °C	9	45	66-67	68	69-70	116	115	190
Boiling point, °C	175	210	245-246	246	Decomposes at 150°C at 16 mm Hg	Sublimes	[a]	293
Density, g/cm^3	1.24 (18/15)[b]	1.38 (60/7)[b]	1.68 (25/25)[c]	1.49 (75/4)[b]	[a]	[a]	[a]	1.98 (22/4)[c]
Vapor pressure, mm Hg[d]	1 (12.1°C)	1 (53°C)	1 (72°C)	1 (76.5°C)	1 (100°C)	[a]	[a]	0.12 (100°C)
Dissociation constant (at 25°C)	3.2×10^{-9}	2.1×10^{-8}	3.7×10^{-8}	3.8×10^{-8}	4.2×10^{-6}	1.1×10^{-7}	3.3×10^{-6}	1.2×10^{-5}
Solubility, g/100 g solvent								
Water	2.85 (20°C)	0.45 (20°C)	Slightly soluble	Slightly soluble	0.01 (25°C)	[a]	Slightly soluble	0.0014 (20°C)
Methanol	[a]	[a]	615	525	319	Very soluble	[a]	57 (20°C)
Diethyl ether	[a]	[a]	[a]	[a]	[a]	[a]		53 (20°C)
Ethanol	>200	Very soluble	[a]	[a]	[a]	[a]	[a]	47 (20°C)
Acetone	Soluble	[a]	615	500	570	[a]	[a]	21 (20°C)
Xylene	[a]	[a]	[a]	[a]	[a]	[a]		14 (20°C)
Benzene	Very soluble	Very soluble	163	113	[a]	[a]	[a]	11 (20°C)
Carbon tetrachloride	[a]	[a]	[a]	[a]	[a]	[a]		2 (20°C)
Ether	>200	Very soluble	[a]	Very soluble	[a]	[a]	[a]	[a]
Chloroform	[a]	Very soluble	[a]	[a]	[a]	[a]	[a]	[a]
Methyl chloride	[a]	Very soluble	[a]	[a]	[a]	[a]	[a]	[a]
Toluene	[a]	[a]	[a]	100	175	[a]	[a]	[a]

[a] No data available.
[b] Density of molten chlorophenol at temperature C_1 compared to density of water at temperature C_2.
[c] No indication was given as to how the determination was made.
[d] Temperature at which vapor pressure was measured is shown parenthetically.

Source: Compiled from Doedens, 1963; Dow Chemical Company, undated; Deichmann and Keplinger, 1963; Bevenue and Beckman, 1967; Weast, 1975.

TABLE A.2.2. SYNTHESIS, USES, AND REACTIONS OF CHLOROPHENOLS

Compound	Commercial synthesis	Principal uses	Important reactions
2-Chlorophenol	Direct chlorination of phenol	Starting material for further chlorination to 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol	Substitution in aromatic ring; phenolic group undergoes etherification, esterification, and condensation reactions and reacts with metallic salts
2,4-Dichlorophenol	Direct chlorination of phenol	Intermediate for production of 2,4-D; ingredient of antiseptics	Substitution in aromatic ring; phenolic group undergoes etherification, esterification, and condensation reactions and reacts with metallic salts
2,4,5-Trichlorophenol	Hydrolysis of 1,2,4,5-tetrachlorobenzene	Intermediate in manufacture of 2,4,5-T and related herbicides; fungicide and bactericide	Substitution in aromatic ring; phenolic group undergoes etherification, esterification, and condensation reactions and reacts with metallic salts
2,4,6-Trichlorophenol	Direct chlorination of phenol	Germicide, particularly for preservation of wood, leather, glue, and textiles; ingredient in preparation of insecticides and soap germicides	Substitution in aromatic ring; phenolic group undergoes etherification, esterification, and condensation reactions and reacts with metallic salts
2,3,4,6-Tetrachlorophenol	Direct chlorination of phenol or chlorination of lower chlorophenols[a]	Fungicide and bactericide for latex, wood, and leather preservation; insecticide	Phenolic group reacts with metallic salts
Pentachlorophenol	Direct chlorination of phenol or chlorination of lower chlorophenols[a]	Fungicide, chiefly for wood preservation; herbicide and general biocide	Phenolic group reacts with metallic salts

[a] Lower chlorophenols include 2-chlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol.

Source: Compiled from Doedens, 1963; Dow Chemical Company, undated; Freiter and Schneider, 1977.

A.2.4 CHEMICAL REACTIVITY

Chlorophenols undergo several reactions; some of the important ones are listed in Table A.2.2. Substitution readily occurs in the aromatic ring of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol. Further chlorination of 2-chlorophenol results in the formation of 2,4- and 2,6-dichlorophenol; 2,4-dichlorophenol reacts to form 2,4,6-trichlorophenol, and 2,4,6-trichlorophenol is converted to 2,3,4,6-tetrachlorophenol and pentachlorophenol. Bromination of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol yields the monosubstituted and disubstituted derivatives. Direct nitration is possible for 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol to the monosubstituted and disubstituted derivatives. The phenolic group of chlorophenols undergoes interesting reactions which yield important pesticidal and germicidal compounds. For example, the herbicides 2,4-D and 2,4,5-T are synthesized by the etherification of 2,4-dichlorophenol and 2,4,5-trichlorophenol, respectively, with monochloroacetic acid. Metal chlorophenates are prepared easily by reacting the phenolic group with metal salts in the presence of organic solvents. The salt forms of chlorophenols are more soluble in water but are less soluble in organic solvents.

Most of the reactions described can only be performed under controlled laboratory conditions. However, conversion of 2-chlorophenol to 2,4- and 2,6-dichlorophenol and of 2,4-dichlorophenol to 2,4,6-trichlorophenol may occur during the chlorination of drinking water and wastewater. These reactions are of great concern because dichlorophenols have been implicated as the compounds contributing largely to the objectionable phenolic odor and taste of water.

A.2.5 TRANSPORT AND TRANSFORMATION IN THE ENVIRONMENT

The transport and transformation mechanisms of chlorophenols in the atmosphere, soils, and aquatic environment are summarized in Section A.7.

A.2.6 ANALYSIS

Common procedures for sample collection and preservation, sample preparation for analysis, and determination are used for all chlorophenols. Electron-capture gas-liquid chromatography is the method of choice for the separation, identification, and quantification of low levels of chlorophenols found in a wide variety of environmental samples. This rapid and sensitive method provides excellent simultaneous resolution of chlorophenol isomers. The aminoantipyrine colorimetric method, although simple and rapid, lacks specificity and is limited to gross determination of chlorophenols or to a single isomer present in a relatively high concentration.

SECTION A.2

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A.3 BIOLOGICAL ASPECTS IN MICROORGANISMS

A.3.1 BACTERIA

A.3.1.1 Metabolism

2-Chlorophenol and 2,4-dichlorophenol are amenable to metabolism by bacteria found in soil and aquatic environments. Phenol-adapted bacteria isolated from soil rapidly metabolize 2-chlorophenol to produce 3-chlorocatecol (3-chloro-1,2-dihydroxybenzene). Microbial breakdown proceeds further to include ring cleavage. Soil bacteria capable of growing on and degrading 2,4-dichlorophenoxyacetic acid (2,4-D) are also adapted to metabolism of 2-chlorophenol and 2,4-dichlorophenol. Degradation by these bacteria proceeds through the formation of 3,5-dichlorocatechol to eventually form dicarboxylic acids, acetate, and chloride. Metabolic breakdown of 2-chlorophenol and 2,4-dichlorophenol also occurs in the presence of certain aquatic bacteria, principally those found in activated sludge. The presence of these bacteria is extremely important for the biological treatment of waste generated from the manufacture of chlorophenols and 2,4-D.

Effective microbial degradation of trichlorophenol and tetrachlorophenol has been demonstrated in activated sludge, lagoon effluent, and enrichment cultures. Thus, effective waste treatment of chlorophenol-containing wastewaters likely occurs when appropriate bacterial populations are present. Microorganisms capable of metabolizing trichlorophenols and tetrachlorophenols have not been isolated from soils, nor have degradation pathways been elucidated. More research is warranted to determine whether bacteria capable of metabolizing higher chlorophenols are widespread in the environment.

Although pentachorophenol is the most refractory of the chlorophenol compounds to microbial degradation, bacteria capable of pentachlorophenol metabolism have been found in soil, water, pentachlorophenol-treated wood, and sewage treatment plants exposed to pentachlorophenol-containing effluents. Certain soil bacteria are able to detoxify pentachlorophenol by methylation, forming pentachloroanisole. Other bacterial strains isolated from continuous-flow enrichment cultures are able to metabolize pentachlorophenol quantitatively with the release of chloride, quantitative disappearance of the substrate, and almost quantitative oxygen uptake. Most bacterial strains capable of degrading pentachlorophenol readily either have been isolated from areas where the compound is commonly found or have been artificially developed in the laboratory, utilizing gradual enrichment and acclimation of the bacteria to increasing levels of pentachlorophenol. The extent to which pentachlorophenol-metabolizing bacteria are present in the environment is not known. In most cases, rapid pentachlorophenol metabolism depends on gradual acclimation. Thus, the hazard posed by pentachlorophenol in environments where the compound has not previously been present may be increased.

A.3.1.2 Effects

All chlorophenols possess bactericidal properties; the potency generally increases with the degree of chlorine substitution up to the trichloro derivatives. The tetrachloro isomers are considerably less active than any of the trichloro isomers; pentachlorophenol is less effective than the tetrachloro isomers and is about as effective as phenol itself. However, some of the higher chlorophenols, particularly pentachlorophenol, have enhanced fungicidal activity. The antibacterial action of phenolic compounds involves several steps: adsorption to the bacterial cell wall, inactivation of essential enzymes, and finally lysis and death of the cell. Phenolic compounds bind and denature proteins and, at high concentrations, act as gross protoplasmic poisons which penetrate rapidly and rupture the cell wall. However, chlorophenols are frequently toxic at levels too low to denature proteins. Thus, another mechanism must be operative at these lower concentration levels. Some studies of the mechanism of action of chlorophenols have indicated that cell membrane damage is a primary effect. Association with cellular lipids and subsequent alteration of cell permeability may be responsible for the toxicity of many chlorophenols.

Other mechanisms for toxicity may be operative in the case of higher chlorophenols. Pentachlorophenol and the tetrachlorophenols are potent uncouplers of oxidative phosphorylation, and it is believed that this effect is of major importance in the toxicity of pentachlorophenol (and likely the tetrachlorophenols) to bacteria. However, many bacteria which are susceptible to control by pentachlorophenol depend primarily on anaerobic fermentation as an energy source. Thus, a mechanism for pentachlorophenol toxicity must include an explanation for the sensitivity of anaerobes to this compound. Although the mechanism of this effect on anaerobes is not understood, results have indicated that pentachlorophenol affects the generation of ATP by substrate-level phosphorylation. Additionally, the inhibition of glycolytic enzymes demonstrated in higher animals may be responsible for the effect noted in bacteria, although no information addressing this point was found.

Although chlorophenols are effective microbicides and several of the compounds find primary usage as disinfectants, detailed data on the relative toxicity of chlorophenols to bacteria are not available. Antibacterial effectiveness of higher chlorophenols is presented in Table A.3.1. Unfortunately, bactericidal activities of lower chlorophenols have been described in the literature only in terms of phenol coefficients. Thus, direct comparison of the relative toxicities of the chlorophenols is difficult. Bactericidal activities of lower chlorophenols and of 2,4,6-trichlorophenol are presented in Table A.3.2. The phenol coefficient measures, under identical conditions, the highest dilution of the test disinfectant effective to kill in 10 min relative to phenol. Thus, disinfectants with phenol coefficients greater than 1 are more potent. Because the data in Tables A.3.1 and A.3.2 cannot be compared directly, only generalizations are possible in assessing the relative antibacterial effectiveness of the compounds. In general, antibacterial effectiveness apparently improves with increased chlorine substitution up to the trichloro derivatives.

TABLE A.3.1. ANTIBACTERIAL EFFICIENCIES OF THE HIGHER CHLOROPHENOLS

Compound	Test organism	Chlorophenol concentration for complete inhibition (mg/liter)	Source
Sodium 2,4,5-trichlorophenate	*Pseudomonas aeruginosa*	300-400	Dow Chemical Company, 1969*a*
	Enterobacter aerogenes	25-50	Dow Chemical Company, 1969*a*
	Bacillus subtilis	10-25	Dow Chemical Company, 1969*a*
	Flavobacterium arborescens	10-25	Dow Chemical Company, 1969*a*
2,4,5-Trichlorophenol	*Aerobacter aerogenes*	20	Walko, 1972
	Bacillus mycoides	15	Walko, 1972
2,3,4,6-Tetrachlorophenol	*Aerobacter aerogenes*	400	Walko, 1972
	Bacillus mycoides	7	Walko, 1972
Pentachlorophenol	*Bacillus cereus* var. *mycoides*	5-10	Dow Chemical Company, undated
	Bacillus subtilis	50-100	Dow Chemical Company, undated
	Escherichia coli	250-500	Dow Chemical Company, undated
	Pseudomonas aeruginosa	1000-2500	Dow Chemical Company, undated
	Enterobacter aerogenes	500-1000	Dow Chemical Company, undated
	Streptomyces griseus	5-10	Dow Chemical Company, undated
	Flavobacterium arborescens	2.5-5	Dow Chemical Company, undated

TABLE A.3.2. BACTERICIDAL ACTIVITIES OF
HALOGENATED PHENOLS

Compound	Phenol coefficient at 37°C	
	Salmonella typhosa	*Staphylococcus aureus*
Phenol (unsubstituted)	1.0	1.0
2-Chlorophenol	3.6	3.7
4-Chlorophenol	3.9	4.6
2,4-Dichlorophenol	13.3	12.7
2,4,6-Trichlorophenol	23.0	25.0
4-Methyl-2-chlorophenol	17.2	15.7
4-Ethyl-2-chlorophenol	6.3	7.5
4-*n*-Butyl-2-chlorophenol	86.0	94.0
4-*n*-Heptyl-2-chlorophenol	16.7	375.0
2-Methyl-4-chlorophenol	28.6	34.4
2-Ethyl-4-chlorophenol	12.5	12.5
2-*n*-Butyl-4-chlorophenol	146.0	257.0
2-*n*-Heptyl-4-chlorophenol	20.0	1500.0

Source: Compiled from Sykes, 1965, Table 33, p. 314, and Baker, Schumacher, and Roman, Tables 26.1 and 26.2, p. 632. Data collected from several sources. Reprinted by permission of the publishers.

In view of the potent antimicrobial activity of chlorophenols, any of these compounds may pose a threat to sewage treatment plants which have not been previously exposed to them. On the other hand, sewage treatment plants regularly exposed to chlorophenols can probably be operated safely with adequate degradation of chlorophenols. In many cases, pentachlorophenol does not exhibit any detectable biochemical oxygen demand (BOD). Because BOD determinations are commonly used in assessing the effectiveness of sewage treatment processes, pentachlorophenol (in the absence of pentachlorophenol-adapted bacteria) will not be detected as a component of the measured BOD, and the compound could conceivably be released from the facility in the absence of other diagnostic tools for its determination.

A.3.2 FUNGI

A.3.2.1 Metabolism

Oxidases (lactase, tyrosinase, and peroxidase) found in white rot fungi are believed to be involved in the degradation of chlorophenols. Most of the information regarding fungal metabolism of chlorophenols is derived from studies of musty taint in broiler chickens. Musty taint results from the uptake of anisoles (formed by methylation of chlorophenols in poultry litter) by chickens, causing an unappetizing taint in the chicken meat. 2,4,6-Trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol are methylated readily by fungi present in poultry litter. Removal of chlorophenols from broiler house litter, whether by methylation or by another mechanism, probably involves many different species of fungi and more than one metabolic route. The degradation of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol by fungi is not well documented. It is believed that fungal metabolism through methylation probably occurs if appropriate fungal strains are present. The mechanism of chlorophenol detoxification by fungi is not well understood. Methylation of the compound may be a primary step in detoxification, and production of compounds such as quinones, chloranil, and tetrachlorobenzoquinone may result from further metabolism of the compounds. The extent to which fungal metabolism contributes to dissipation of chlorophenols from the environment is not known, and further research is warranted.

A.3.2.2 Effects

Information on the mechanism of action of chlorophenols on fungi is not available. Toxicity of chlorophenols to fungi may result from mechanisms similar to those discussed for bacteria (Section A.3.1.2). Reported effects on oxidative phosphorylation, particularly with respect to higher chlorophenols, may also be important in the toxicity of these compounds to fungi. 2,4,5-Trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol are commonly used as preservatives and general purpose microbicides. The antifungal activity of these compounds has been documented; their toxicity to various species of fungi is presented in Table A.3.3. No information on the antifungal activity of 2-chlorophenol and 2,4-dichlorophenol was found.

A.3.3 ALGAE

A.3.3.1 Metabolism

No information is available on the metabolism of chlorophenols by algae.

A.3.3.2 Effects

Chlorophenols are toxic to algae. Their toxicity to algae has been studied under laboratory conditions and expressed in terms of effects on chlorophyll synthesis (Table A.3.4). Table A.3.5 lists the toxicity constants for chlorophenols. Increasing the number of chlorine atoms on the aromatic rings of chlorophenols enhances their toxicity. The trichlorophenol isomers, 2,4,5- and 2,4,6-trichlorophenol, are at least 100 times more toxic to algae than either 2-chlorophenol or 2,4-dichlorophenol. Pentachlorophenol is extremely toxic to algae and is the most toxic compound among the ten halogenated phenols tested for their effects on chlorophyll synthesis. Even at concentrations as low as 1.5 mg/liter,

TABLE A.3.3. ANTIFUNGAL EFFICIENCIES OF CHLOROPHENOLS

Compound	Test organism	Chlorophenol concentration for complete inhibition (mg/liter)	Source
Sodium 2,4,5-trichlorophenate	*Rhizopus nigricans*	40-55	Dow Chemical Company, 1969a
	Rhizoctonia solani	4-6	Dow Chemical Company, 1969a
	Chaetomium globosum	8-17	Dow Chemical Company, 1969a
	Hormiscium gelatinosum	11-28	Dow Chemical Company, 1969a
	Aspergillus No. 29	6-11	Dow Chemical Company, 1969a
	Polyporus tulipiferae	8-11	Dow Chemical Company, 1969a
	Aspergillus flavus	6-11	Dow Chemical Company, 1969a
	Lenzites trabea	4-6	Dow Chemical Company, 1969a
	Ceratostomella pilifera	6-11	Dow Chemical Company, 1969a
	Trichophyton interdigitale	2-40	Dow Chemical Company, 1969a
	Trichophyton rosaceum	11-22	Dow Chemical Company, 1969a
	Aspergillus niger	75	Mason, Brown, and Minga, 1951
2,4,5-Trichlorophenol	*Aspergillus niger*	15	Walko, 1972
	Penicillium expansum	7	Walko, 1972
	Rhizopus nigricans	35-50	Dow Chemical Company, 1969[b]
	Rhizoctonia solani	4-5	Dow Chemical Company, 1969[b]
	Chaetomium globosum	8-15	Dow Chemical Company, 1969[b]
	Hormiscium gelatinosum	10-25	Dow Chemical Company, 1969[b]
	Aspergillus No. 29	5-10	Dow Chemical Company, 1969[b]
	Polyporus tulipferae	7-10	Dow Chemical Company, 1969[b]
	Aspergillus flavus	5-10	Dow Chemical Company, 1969[b]
	Lenzites trabea	4-5	Dow Chemical Company, 1969[b]
	Ceratostomella pilifera	5-10	Dow Chemical Company, 1969[b]
	Trichophyton interdigitale	2-4	Dow Chemical Company, 1969[b]
	Trichophyton rosaceum	10-20	Dow Chemical Company, 1969[b]
2,4,6-Trichlorophenol	*Trichoderma viride*	7[a]	Blackman, Parke, and Garton, 1955
Sodium 2,3,4,6-tetrachlorophenate	*Aspergillus niger*	310	Mason, Brown, and Minga, 1951
2,3,4,6-Tetrachlorophenol	*Aspergillus niger*	20	Walko, 1972
	Penicillium expansum	30	Walko, 1972
	Trichoderma viride	0.8[a]	Blackman, Parke, and Garton, 1955
Pentachlorophenol	*Trichoderma viride*	25-50	Dow Chemical Company, undated
	Trichoderma sp.	10-25	Dow Chemical Company, undated
	Ceratocystis pilifera	5-10	Dow Chemical Company, undated
	Polyporus tulipiferae	<1	Dow Chemical Company, undated
	Rhizopus stolonifer	1	Dow Chemical Company, undated
	Lenzites trabea	1-2.5	Dow Chemical Company, undated
	Ceratocystis ips	10-25	Dow Chemical Company, undated
	Chaetomium globosum	1-2.5	Dow Chemical Company, undated
	Aspergillus niger	10-25	Dow Chemical Company, undated

[a] Value for 50% inhibition of growth.

[b] Dow Chemical Company purified grade, proprietary name Dowicide EC-7.

TABLE A.3.4. EFFECT OF CHLOROPHENOLS ON CHLOROPHYLL SYNTHESIS BY *CHLORELLA PYRENOIDOSA*

Compound	Concentration (mg/liter)	Chlorophyll (mg/liter)			
		After 0 day	After 1 day	After 2 days	After 3 days
2-Chlorophenol	Control	9.6	24.8	74.6	66.8
	10	9.6	25.6	76.8	64.8
	50	9.6	16.8	40.0	52.0
	100	9.6	10.4	28.0	44.0
	250	9.6	9.6	8.4	8.0
	500	9.6	6.4	2.4	1.2
3-Chlorophenol	Control	9.6	24.8	74.6	66.8
	10	9.6	24.8	76.0	64.8
	50	9.6	18.4	22.8	32.0
	100	9.6	10.0	9.4	8.4
	250	9.6	6.0	3.2	1.2
	500	9.6	0	0	0
4-Chlorophenol	Control	9.6	24.8	74.6	66.8
	10	9.6	24.6	75.6	66.8
	50	9.6	20.0	25.2	36.0
	100	9.6	11.6	12.8	13.6
	250	9.6	6.8	4.4	2.0
	500	9.6	2.0	0	0
2,4-Dichlorophenol	Control	10.4	24.8	77.0	64.8
	1	10.4	25.6	46.8	60.0
	10	10.4	12.8	36.6	50.2
	25	10.4	9.6	15.6	24.0
	50	10.4	8.0	11.6	12.8
	100	10.4	5.0	1.6	0
2,4,5-Trichlorophenol	Control	10.4	24.8	77.0	65.8
	1.0	10.4	20.0	52.8	56.8
	2.5	10.4	16.0	18.4	26.4
	5.0	10.4	12.2	9.6	8.0
	7.5	10.4	8.8	6.2	2.4
	10.0	10.4	6.2	1.6	0
2,4,6-Trichlorophenol	Control	10.4	24.8	77.0	64.8
	1.0	10.4	24.8	74.4	58.4
	2.5	10.4	18.4	26.0	32.0
	5.0	10.4	15.6	12.8	9.6
	7.5	10.4	11.6	7.2	4.4
	10.0	10.4	8.8	4.4	0
Pentachlorophenol	Control	9.7	26.4	77.6	68.8
	0.0008	9.7	11.6	23.6	44.4
	0.0015	9.7	7.0	7.0	20.0
	0.0038	9.7	4.6	5.2	6.8
	0.0075	9.7	2.4	0	0

Source: Adapted from Huang and Gloyna, 1967, Table 3-9, p. 56, and Table 3-10, p. 61.

TABLE A.3.5. TOXICITY CONSTANTS OF CHLOROPHENOLS TO *CHLORELLA PYRENOIDOSA*

Organic compound	Toxicity constant ($\times 10^{-3}$)	Phenol coefficient	Concentration tested showing no toxicity (mg/liter)
Phenol	2.99	1.00	
2-Bromophenol	8.88	2.97	10.0
3-Bromophenol	20.53	6.85	10.0
4-Bromophenol	18.75	6.28	10.0
2-Chlorophenol	7.18	2.40	10.0
3-Chlorophenol	18.12	6.06	10.0
4-Chlorophenol	15.28	5.11	10.0
2,4-Dichlorophenol	32.70	10.90	1.0
2,4,5-Trichlorophenol	472.00	158.00	1.0
2,4,6-Trichlorophenol	412.00	138.00	1.0
Pentachlorophenol	656,000.00	222,000.00	

Source: Compiled from Huang and Gloyna, 1967.

pentachlorophenol inhibited chlorophyll synthesis for two days. Pentachlorophenol and sodium pentachlorophenate are effective algicides and have been used commercially for this purpose. The potential for serious effects on waste stabilization ponds (where the healthy growth of algae is important in waste treatment) may be jeopardized by the presence of higher chlorophenols (trichlorophenols and pentachlorophenol). Tetrachlorophenols have not been tested for their effect on algae, but their toxicity may be similar to that of pentachlorophenol.

SECTION A.3

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A.4 BIOLOGICAL ASPECTS IN PLANTS

A.4.1 METABOLISM

A.4.1.1 Uptake and Absorption

Data on the uptake and absorption of chlorophenols by vascular plants are scant. 2,4-Dichlorophenol is rapidly absorbed by the roots of oats and soybeans grown in nutrient solution or soil containing 2,4-dichlorophenol. More 2,4-dichlorophenol is absorbed when the plants are grown in nutrient solution than in soil; absorption depends on such factors as plant species, age, growth, and media composition. Pentachlorophenol is absorbed readily by vascular plants. The compound is absorbed following foliar application to sugar cane and cotton plants, and rapid absorption by the roots occurs in sugar cane grown in nutrient solutions containing pentachlorophenol. Data on other chlorophenols are not available.

A.4.1.2 Transport and Distribution

Small, detectable quantities of 2,4-dichlorophenol are transported to shoots of oats and soybeans following root absorption from nutrient solutions or soils. The substance is not translocated to the grain of oat plants; however, small quantities are found in soybean seeds following absorption. Oats appear capable of concentrating 2,4-dichlorophenol from nutrient solution by a factor of 9; however, no concentration effect is seen when oats are grown in soil containing 2,4-dichlorophenol, nor is concentration seen when soybeans are grown in nutrient solution or soil. 2,4-Dichlorophenol appears to be relatively immobile following foliar application to soybeans.

Transport of pentachlorophenol in vascular plants depends on species characteristics. Very little pentachlorophenol is transported in sugar cane grown in nutrient solution containing pentachlorophenol, although a small quantity of the substance is found in the stalks and suckers. Similarly, pentachlorophenol remains at the site of foliar application in sugar cane until the leaves abscise and the compound is lost from the plant. Conversely, some pentachlorophenol is translocated in cotton plants. Detectable levels of pentachlorophenol were found in cotton seed kernels of bolls which were closed at the time the plants were sprayed with a solution containing pentachlorophenol, indicating translocation from the exterior of the bolls to the kernels. Furthermore, some pentachlorophenol translocation occurred within cotton seedlings following foliar application.

Based on the available data, contamination of food crops with chlorophenols present in soil is not likely. Absorption by roots, on the other hand, may pose a problem in the cultivation of roots and tubers in soils contaminated with chlorophenols. Furthermore, limited data indicate that pentachlorophenol may be transported within cotton plants following foliar application.

A.4.1.3 Biotransformation

Plants are capable of metabolizing a number of chlorophenols. Glycoside formation (conjugation) and subsequent immobilization of 2-chlorophenol has been demonstrated in the roots of tomato plants grown in sand cultures containing the compound. The further fate of the conjugate identified as β-*O*-chlorophenyl gentiobioside is not known.

Glycoside formation and immobilization of 2,4-dichlorophenol in strawberry leaves has also been reported. The appearance and subsequent dissipation of 2,4-dichlorophenol from rice plants and soils following application of the herbicide dichlorophenoxyacetic acid (2,4-D) has been demonstrated. As the plants matured, 2,4-dichlorophenol residues declined in the plants, and no 2,4-dichlorophenol was detected at maturity. The metabolic fate of the compound is not known.

Both free 2,3,4,6-tetrachlorophenol and unidentified tetrachlorophenol conjugates have been reported in lettuce plants following treatment with lindane.

The metabolism of pentachlorophenol in sugar cane roots has been demonstrated, but it is not known whether conjugation or another mechanism is responsible for the degradation. Based on evidence presented for other chlorophenols, conjugate formation of pentachlorophenol may occur in vascular plants, but the pathways responsible for further metabolism have not been elucidated.

A.4.1.4 Elimination

Chlorophenols may be dissipated from plants in four ways: (1) volatilization from leaf surfaces or stems, (2) metabolism with subsequent excretion of metabolites, (3) excretion through the roots, and (4) loss through abscission of leaves and other plant parts.

The substantial vapor pressure of chlorophenols indicates that volatilization provides a route of elimination from vascular plants. Volatilization has been proposed as a mechanism for the elimination of 2,4-dichlorophenol from soybean plants receiving foliar applications and for the loss of pentachlorophenol following application to sugar cane.

Excretion of the herbicide 2,4-D through the roots of vascular plants has been demonstrated, and it is proposed that 2,4-dichlorophenol (a major metabolite of the herbicide) may also be excreted in this manner. Excretion of pentachlorophenol from the roots of sugar cane following absorption has also been shown.

Chlorophenols present in vascular plants may also be eliminated by abscission of leaves and other plant parts if the compound is not transported or removed rapidly from the plant in other ways. Data indicate that chlorophenols are relatively immobile compounds and that elimination by this mechanism may be important.

A.4.2 EFFECTS

A.4.2.1 Physiological or Biochemical Role

No physiological or biochemical requirement for chlorophenols by vascular plants has been documented.

A.4.2.2 Toxicity

A.4.2.2.1 Mechanisms of Action – The mechanism of the phytotoxic action of chlorophenols is not well understood. Both 2-chlorophenol and 2,4-dichlorophenol are capable of inhibiting the enzyme catalase. The contribution of this enzymatic effect to the phytotoxicity of chlorophenols is not known. Pentachlorophenol is capable of uncoupling oxidative phosphorylation in both plants and animals. This mechanism appears to be the most likely explanation for the clearly demonstrable toxicity of pentachlorophenol. It seems likely – based on evidence with other organisms – that other chlorophenols (particularly the tetrachlorophenols) share this property with pentachlorophenol.

A.4.2.2.2 General Toxicity – No documented cases of 2-chlorophenol phytotoxicity were found in the literature. Only one report documented the phytotoxicity of 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol. The concentrations of these compounds required to obtain 50% mortality in the aquatic plant *Lemna minor* are given in Table A.4.1. Increasing chlorine substitution appears to enhance toxicity. The data in Table A.4.1 have been corrected for disassociation effects at the experimental pH of 5.1. Logarithms of these data were plotted against the logarithms of the solubility of chlorophenols (Figure A.4.1); the linear relationship indicates that the physiological effect (toxicity) is increased as the pH of the medium approaches

TABLE A.4.1. CONCENTRATION OF CHLOROPHENOLS NECESSARY FOR 50% MORTALITY OF *LEMNA MINOR*

Compound	Concentration (moles/liter)	Concentration (mg/liter)
Phenol	1.6×10^{-2}	1500
4-Chlorophenol	2.2×10^{-3}	283
2,4-Dichlorophenol	3.6×10^{-4}	59
2,4,6-Trichlorophenol	3.0×10^{-5}	6.0
2,4,5-Trichlorophenol	8.4×10^{-6}	1.7
2,3,4,6-Tetrachlorophenol	2.6×10^{-6}	0.61
Pentachlorophenol	7.1×10^{-7}	0.19

Source: Adapted from Blackman, Parke, and Garton, 1955*a*, Table I, p. 50. Reprinted by permission of the publisher.

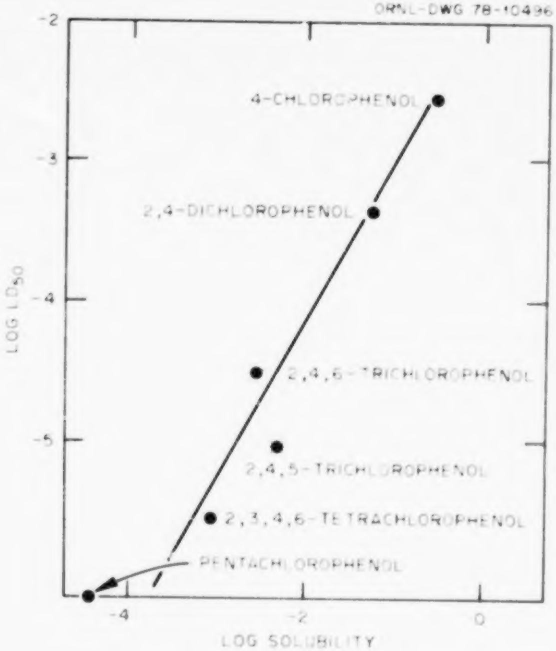

Figure A.4.1. Relationship between the logarithms of the solubilities of chlorophenols and the logarithms of the LD$_{50}$ in *Lemna minor*. Source: Adapted from Blackman, Parke, and Garton, 1955*b*, Figure 2, p. 63. Reprinted by permission of the publisher.

the p*K* of the chlorophenol. It is evident that pH is of major importance in assessing chlorophenol toxicity to aquatic plants. No other information on the phytotoxic effects of the lower chlorophenols is available. However, pentachlorophenol is clearly phytotoxic and has been used as a herbicide. Deaths of terrestrial plants grown in pentachlorophenol-treated containers or near pentachlorophenol-treated fence posts have been reported. When direct plant contact with pentachlorophenol was not evident, pentachlorophenol in the air was thought to be responsible for plant injury. Pentachlorophenol also has been used as an effective general herbicide, and caution must be exercised when the compound is used in proximity to crops. Pentachlorophenol was the most toxic of the chlorophenols when tested with the aquatic plant *Lemna minor* (Table A.4.1, Figure A.4.1), and contamination of waterways with pentachlorophenols poses a potentially serious hazard to aquatic flora.

A.4.2.2.3 Mitotic Effects — Several chlorophenols affect mitosis in vascular plants. 2,4-Dichlorophenol treatment of the European broad bean (*Vicia faba*) caused an increased frequency of mitotic anomalies in pollen mother cells. This effect depended on plant age, but decreased plant yield was noted through the second generation. 2,4,5-Trichlorophenol did not affect the frequency of mitotic anomalies when tested in the same system.

Pentachlorophenol is also capable of increasing the number of mitotic abnormalities in plants treated with the compound. A positive effect of pentachlorophenol on the number of mitotic anomalies, a considerable decrease in the mitotic index of a root preparation of *Vicia faba*, and an increase in the number of mitotic abnormalities in a root tip preparation of water hyacinth (*Eichhornia crassipes*) have been reported. Other chlorophenols have not been tested for the presence or absence of this effect.

SECTION A.4

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A.5 BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

A.5.1 BIOLOGICAL ASPECTS IN BIRDS AND MAMMALS

A.5.1.1 Metabolism

A.5.1.1.1 Uptake and Absorption -- No information is available on the direct absorption of lower chlorophenols by birds or domestic mammals. Ample evidence exists, however, that pentachlorophenol is absorbed by inhalation, through the skin, or following ingestion and that it produces toxic symptoms. It is speculated that tetrachlorophenols share similar properties with pentachlorophenol because their chemical structures are similar and the mechanisms of their toxicity appear similar, but no definitive data are available. The possible routes of uptake of lower chlorophenols are speculative.

A.5.1.1.2 Transport and Distribution — Following entry into the body, chlorophenols are transported mainly by the blood. Pentachlorophenol is known to be transported by this route, but direct data on the mode of transport of other chlorophenols in domestic mammals and birds are not available.

Data on the tissue distribution of chlorophenols in birds and mammals are sketchy. Most data on lower chlorophenols are derived from experiments tracing the metabolic fate of pesticides in the organisms. Many pesticides and commonly used organic chemicals produce chlorophenols as metabolites. The applicability of tissue distribution data following administration of precursor compounds as opposed to direct administration of a chlorophenol is not understood. However, indirect information sheds some light on tissue distribution. 2,4-Dichlorophenol has been detected in the liver and kidneys of sheep and cattle following dichlorophenoxyacetic acid (2,4-D) administration, but none was found in the muscles or fat. 2,4-Dichlorophenol was detected in liver tissue and egg yolk from chickens dosed with nemacide [*O*-(2,4-dichlorophenyl)*O*,*O*-diethyl phosphorothioate] but was not found in muscle or fat. Following administration of high doses of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to cows, 2,4,5-trichlorophenol was detected in milk and cream from the animals. Tissue levels of 2,4,5-trichlorophenol were not determined. High levels of pentachlorophenol were found in the kidneys of swine fed pentachlorophenol, but very little was found in fat, liver, or muscle.

Much of the available information on the transport and distribution of chlorophenols has been derived from experiments conducted with laboratory animals. This information is summarized primarily in Section A.6.1.2; the reader is referred to that section for information obtained by extrapolating from laboratory animals to domestic animals and wildlife.

A.5.1.1.3 Biotransformation — Information on the metabolism of chlorophenols is mainly derived from experimental animals (Section A.6.1.3).

A.5.1.1.4 Elimination — No information on the elimination of lower chlorophenols from domestic animals or wildlife is available. It seems likely that chlorophenols are excreted primarily in the urine. Pentachlorophenol is rapidly excreted in the urine, but some residues have been detected in the feces of sheep and cattle following administration of the compound. Pentachlorophenol is the most lipophilic of the compounds examined in this report. Since elimination of pentachlorophenol from the body occurs rapidly through the urine, the more soluble, less lipophilic, lower chlorophenols would probably be excreted even more rapidly.

A.5.1.2 Effects

A.5.1.2.1 Physiological or Biochemical Role — No evidence indicates that any chlorophenols play a physiological or biochemical role in domestic animals or birds, and it is unlikely that any nutritional requirements for the compounds exist.

A.5.1.2.2 Toxicity — Of all chlorophenols, only pentachlorophenol has a documented toxic effect on domestic animals and wildlife. 2,4,5-Trichlorophenol was fed to cattle at levels up to 159 mg/kg body weight for 78 days, and no effect on the animals was noted at any dose level.

The toxicity of pentachlorophenol to many animals — including swine, cattle, sheep, cats, rabbits, and other laboratory animals — is well documented. Both local effects (irritation and necrosis of the skin) and systemic effects, including death, have been reported. Constant direct cutaneous exposure to pentachlorophenol-treated wood has caused mortality in swine. However, present evidence does not indicate a direct hazard to domestic or wild animals from pentachlorophenol-treated wood unless they are in constant contact with the wood. Nonetheless, more information is needed to demonstrate whether chronic exposure to low levels of pentachlorophenol poses a subtle hazard to the health of domestic and wild animals. Levels of pentachlorophenol in the tissues of domestic animals exposed to pentachlorophenol rarely have been documented. Humans would be exposed to pentachlorophenol if meat were contaminated; therefore, further research in this area is highly recommended. Another hazard may be posed by the use of pentachlorophenol as a herbicide or molluscicide. Extensive losses of wildlife, including fish, frogs, snails, and birds, were attributed to molluscicidal application of pentachlorophenol in Surinam. Sublethal levels of the compound were also detected in animals which were not killed; the effect of these levels is not known. Chlorophenols other than pentachlorophenol are not used directly as herbicides and their use as preservatives is limited.

Anisoles — metabolites of chlorophenols — are responsible for the presence of a musty taint occasionally associated with broiler chickens. 2,4,6-Trichloroanisole, 2,3,4,6-tetrachloroanisole, and pentachloroanisole, which are formed from the corresponding chlorophenols in poultry litter, are responsible for this phenomenon. Because tainted chickens are unappetizing, the hazards to humans that may be associated with ingestion of the anisoles are minimized, and the problem becomes primarily an economic issue. Rearing chickens on litter which does not contain chlorophenols or removal of chickens from tainted litter several weeks prior to slaughter usually resolves the problem. The question of whether or not chlorophenols are absorbed directly by the chickens along with the anisoles warrants further research.

A.5.2 BIOLOGICAL ASPECTS IN FISH AND OTHER AQUATIC ORGANISMS

Data on the metabolism of lower chlorophenols and their effects on aquatic organisms are extremely scarce. The toxic levels of these compounds for various aquatic organisms are available, but the great bulk of information relates to pentachlorophenol. Information on the metabolism and toxicity of pentachlorophenol to aquatic organisms found in Section D.5.2 will not be duplicated in this section; only a brief overview is presented below.

A.5.2.1 Metabolism

A.5.2.1.1 Uptake and Absorption — Uptake of chlorophenols by aquatic organisms can be inferred from the documented toxicity of these compounds (Section A.5.2.2). Fish and other aquatic organisms absorb chlorophenols through the gills, the gastrointestinal tract, or directly through the body surfaces. Most available toxicity data do not deal with the route of uptake of the compound, and rates of chlorophenol uptake are frequently speculative. No direct data on the route or rates of uptake of lower chlorophenols by aquatic organisms were found. Pentachlorophenol is rapidly removed from the surrounding water by the rainbow trout, short-necked clam, eel, and goldfish. This rapid uptake is followed by excretion of pentachlorophenol, detoxification of pentachlorophenol by conjugation, subsequent excretion of the detoxified conjugate, and accumulation, particularly in the fat of the eel.

A.5.2.1.2 Transport and Distribution — Transport and distribution of lower chlorophenols following absorption by aquatic organisms have not been studied. However, considerable evidence on the transport and distribution of pentachlorophenol in aquatic organisms is available. Following absorption of pentachlorophenol by the short-necked clam, high levels of the compound appear in the bojanus' organ, the liver, and the digestive tract. Pentachlorophenol appears to be transported primarily by the blood in aquatic organisms. High levels of pentachlorophenol were detected in the liver and muscle of eels dosed with the compound. Goldfish accumulate large quantities of pentachlorophenol in the gallbladder, and measurable amounts are also detected in the gills, kidney, and hepatopancreas.

A.5.2.1.3 Biotransformation — No information on the metabolism of lower chlorophenols in aquatic organisms is available. Pentachlorophenol appears to be detoxified by the short-necked clam and the goldfish; detoxification is accomplished by a similar mechanism in both cases. Sulfate conjugation and subsequent excretion have been demonstrated following uptake of pentachlorophenol by these organisms. It has been suggested that pentachlorophenol is detoxified in fish following transfer to the hepatopancreas and that the metabolites, in part, are transported to the gallbladder and bile. The extent to which conjugate formation occurs as a detoxification mechanism in other aquatic organisms is not known.

A.5.2.1.4 Elimination — Routes and rates of elimination of lower chlorophenols from aquatic organisms are not known. Pentachlorophenol is excreted as a sulfate ester from the short-necked clam and the goldfish following uptake. There are apparently at least two rates of excretion by goldfish. Pentachlorophenol excretion is initially rapid; approximately 80% of the pentachlorophenol content of the fish is excreted within 20 hr. Excretion of the remaining 20% is much slower. The hepatopancreas and the gallbladder are believed to play important roles in the excretion of pentachlorophenol by goldfish; the slow excretion seen after the initial rapid excretion stage may result partially from the storage of the compound in the gallbladder. The gallbladder may also play an important role in the excretion of pentachlorophenol by mammals.

A.5.2.2 Effects

A.5.2.2.1 Physiological or Biochemical Role — No evidence indicates that any chlorophenols play a physiological or biochemical role in aquatic organisms, and it is unlikely that any nutritional requirement for the compounds exists.

A.5.2.2.2 Toxicity

A.5.2.2.2.1 Mechanism of action — The mechanism of toxic action of chlorophenols on aquatic organisms is not well understood. 2-Chlorophenol inhibits oxygen uptake in in vitro systems utilizing various fish tissue preparations, but the contribution of this effect to the toxicity of 2-chlorophenol in intact organisms is unknown. The biochemical mechanism of pentachlorophenol toxicity probably derives from its ability to uncouple oxidative phosphorylation and, at higher concentrations, to inactivate glycolytic enzymes. This effect has been well documented in experimental mammals, and it is believed that the same effect is responsible for the toxic action of the compound in aquatic organisms.

A.5.2.2.2.2 Acute toxicity — Information on the toxicity of lower chlorophenols to aquatic organisms is limited. Dosages and effects on various aquatic organisms are presented in Table A.5.1. Toxicity data on 2,4,5-trichlorophenol and the tetrachlorophenols were not found. The acute toxicity of pentachlorophenol to aquatic organisms has been well studied; a few typical values are listed in Table A.5.1. The reader is referred to Section D.5.2.2.2 for data on toxicity of pentachlorophenol to a large number of fish and invertebrate species. Although it is difficult to draw conclusions from the limited data in Table A.5.1, the toxicity of chlorophenols to aquatic organisms likely increases with increasing chlorination of the parent phenol. Pentachlorophenol is by far the most toxic compound for which data are available. Fish kills have been reported when pentachlorophenol reaches waterways either from pentachlorophenol-contaminated effluents, spillage of the concentrated compound, or herbicide use. Pentachlorophenol concentrations resulting in

TABLE A.5.1. TOXICITY OF CHLOROPHENOLS TO AQUATIC ORGANISMS

Compound	Test organism	Level of chlorophenol (mg/liter)	Parameter measured	Source
2-Chlorophenol	Bluegill (*Lepomis macrochirus*)	8.1	48-hr TL_m[a]	Lammering and Burbank, 1960
	Rainbow trout (*Salmo gairdnerii*)	2.7	48-hr TL_m	Sletten and Burbank, 1972
	Daphnia magna	7.4	48-hr TL_m	Kopperman, Carlson, and Caple, 1974
2,4-Dichlorophenol	Fertilized sea urchin eggs (*Arbacia punctulata*)	10.3	Decreased rate of cell division and increased O_2 consumption	Clowes, 1951
	Daphnia magna	2.6	48-hr TL_m	Kopperman, Carlson, and Caple, 1974
2,4,6-Trichlorophenol	Fathead minnow (*Pimephales promelas*)	0.6	96-hr TL_m	Manufacturing Chemists Association, 1972
	Aquatic microfauna (particularly stalked ciliates)	4.0	Decrease in total number of organisms	Manufacturing Chemists Association, 1972
	Fertilized sea urchin eggs (*Arbacia punctulata*)	6.2	Decreased rate of cell division and increased O_2 consumption	Clowes, 1951
Pentachlorophenol	Bluegill (*Lepomis macrochirus*)	0.03	48-hr TL_m	Inglis and Davis, 1973
	"Warasubo" fish (*Odontamblyopus rubicundus*)	0.25	48-hr TL_m	Tomiyama and Kawabe, 1962
	Channel catfish (*Ictalurus punctatus*)	0.46	48-hr TL_m	Clemens and Sneed, 1959
	Shellfish (*Conchocelis* sp.)	0.80	No toxic effect	Tomiyama, Kobiyashi, and Kawabe, 1962a
	Shellfish (*Venerupis philippinarum*)	0.10	100% mortality	Tomiyama, Kobiyashi, and Kawabe, 1962b
	Cladocera (*Daphnia pulex*)	5.0	No toxic effect	Goodnight, 1942
	Crayfish (*Cambarus virilis*)	9.5	No toxic effect	Goodnight, 1942

[a] TL_m — Median tolerance limit.

50% mortality of fish depend on the species and range from a low of 30 µg/liter for the bluegill to as high as 460 µg/liter for the channel catfish and 600 µg/liter for the top minnow (*Fundulus notatus*). Some aquatic invertebrates, including several species of commercial shellfish, are as sensitive to pentachlorophenol as fish. On the other hand, many aquatic invertebrates can survive concentrations as high as 5 to 10 mg/liter with no apparent toxic effects. Of all chlorophenols, pentachlorophenol clearly poses the greatest potential hazard to aquatic flora and fauna.

A.5.2.2.2.3 Chronic toxicity — The lethal dose values in Table A.5.1 are at best a poor indication of the true environmental significance of chlorophenols. Although dramatic effects such as massive fish kills are seen readily and are aesthetically objectionable, more subtle effects may occur in aquatic communities exposed to chlorophenols. Such effects may decrease the ability of some species to reproduce, thereby affecting the survival of a species or perhaps the survival and integrity of entire biological communities. Data addressing the chronic or sublethal effects of lower chlorophenols are scant. 2,4-Dichlorophenol and 2,4,6-trichlorophenol adversely affected the development of fertilized sea urchin eggs at concentrations of 10.3 mg/liter and 6.2 mg/liter respectively.

Data suggest that sublethal levels of pentachlorophenol subtly affect aquatic organisms. Pentachlorophenol is lethal to developing trout embryos and alevins at a concentration of 40 µg/liter. Decreased growth and curtailment of food conversion efficiency in the sockeye salmon reportedly occurs at pentachlorophenol levels of less than 2 µg/liter. Other more serious effects, including renal degeneration, liver damage, and retarded gonadal development, may occur at higher concentrations (500 µg/liter). A large number of metabolic parameters, including the activity of a number of liver enzymes, were affected in eels exposed to 100 µg/liter pentachlorophenol. Other reports indicated severe developmental abnormalities in a number of fish species at pentachlorophenol concentrations greater than 1 mg/liter. It has been suggested that the maximum permissible concentration of pentachlorophenol in water should be 0.1 mg/liter. In view of the adverse effects seen at concentrations as low as 2 µg/liter, this maximum concentration seems far too high. The seriousness of the potential problem is emphasized by data which indicate that pentachlorophenol may bioaccumlate in aquatic organisms living in pentachlorophenol-contaminated water and in other organisms which may be exposed to the compound through food chain interactions. Due to a lack of information, the hazard to aquatic organisms posed by lower chlorophenols cannot be assessed.

A.5.2.2.2.4 Effect of environmental parameters on toxicity — Published toxicity values are valid only for the specific conditions used by individual investigators. Differing environmental conditions can affect the toxicity of chlorophenols to aquatic organisms. For example, the toxicity of pentachlorophenol to aquatic animals is enhanced by decreases in pH and dissolved oxygen content. Pentachlorophenol toxicity is also enhanced by an increase in temperature. The effects of these factors, as well as others which have not been examined, on pentachlorophenol toxicity must be assessed on an individual basis. Another potential hazard in interpreting chlorophenol toxicity is the possible presence of other pollutants — either natural or man-made. Other compounds may either enhance or decrease the toxicity of individual chlorophenols; these synergistic effects in the aquatic environment are poorly understood.

A.5.2.2.2.5 Bioconcentration and biomagnification in aquatic organisms — It is not known whether biomagnification either from the surrounding water or from food chain interactions occurs with any lower chlorophenols. Evidence exists that these processes occur when pentachlorophenol is present in the water. Pentachlorophenol levels in fish taken from a lake receiving discharges of pulp mill effluents were found to be 100- to 1000-fold higher than pentachlorophenol levels in the lake water. The eel, a species with a higher than normal fat content, concentrated pentachlorophenol to the greatest extent. In laboratory experiments, pentachlorophenol levels in goldfish were found to be 1000-fold higher than the concentration in the medium following an exposure time of 120 hr. The high levels of pentachlorophenol found in goldfish probably resulted from direct absorption and biomagnification by the fish. Other species of fish have been shown to concentrate pentachlorophenol, but not to the same extent. High levels found in fish taken from a contaminated lake may have been caused by either direct absorption and concentration of the compound or by food chain biomagnification.

Biomagnification of pentachlorophenol in a simple laboratory six-element food chain has been demonstrated. The model aquatic ecosystem contained algae, snails, daphnia, mosquito larvae, and mosquito fish. At the termination of the experiment the organisms contained higher concentrations of pentachlorophenol than did the medium. The ecological magnification factors (defined as ratio of concentration of pentachlorophenol in the organisms to concentration of pentachlorophenol in the water) for these organisms were: algae, 1.58; mosquito larvae, 16; snail, 131; daphnia, 165; and mosquito fish, 296. Although food chain biomagnification is not shown conclusively by these data, a strong correlation between the trophic status of the organisms and the ecological magnification factors is evident. Data relating to food chain biomagnification in the environment are not available; however, the process may be responsible for some of the high levels of pentachlorophenol detected in aquatic organisms. Although pentachlorophenol is metabolized rapidly by many aquatic organisms, the hazards posed to aquatic ecosystems and eventually to humans must be carefully considered. Evidence exists that pentachlorophenol may persist in fish for as long as two months following exposure to the compound and transfer to clean water. These levels, although low, present the potential for chronic low-level exposure to humans.

The above experiment with the model aquatic ecosystem was also conducted with a number of other commonly used organic compounds. Pentachlorophenol was roughly intermediate among the compounds tested with respect to ecological magnification value. For example, DDT was found to have an ecological magnification factor of 17,000. Food chain hazards associated with DDT have been well documented; hazards posed by pentachlorophenol are probably of a lower degree. Ecological magnification values for hexachlorocyclohexane and chlorobenzene were 1166 and 650 respectively. These values are of interest because pentachlorophenol may be formed following metabolism of these compounds in higher organisms. For example, substantial amounts of pentachlorophenol were formed by members of the model community as a metabolic breakdown product of hexachlorocyclohexane. Thus, levels of pentachlorophenol in waterways may result in part from the degradation of other compounds and, if pentachlorophenol does not reach waterways directly, the compound may be produced by th metabolic processes. Thus, total elimination of pentachlorophenol from waterways may be a formidable, if not impossible, task.

A.5.2.2.2.6 Detection and avoidance reactions — Fish are capable of detecting pentachlorophenol and demonstrate an avoidance reaction to the compound when placed in a pentachlorophenol gradient. Species differences play a major part in the pentachlorophenol level which can be discriminated by fish. The usefulness of this mechanism to the individual organism in avoiding areas of local pentachlorophenol toxicity is questionable because levels of pentachlorophenol which cause chronic toxicity are in many cases below the detectable threshold discrimination of fish. Furthermore, an avoidance reaction may have little usefulness when all or a large portion of the environment of the fish is contaminated with the compound. No evidence suggests that fish are capable of detecting and avoiding other chlorophenols.

SECTION A.5

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A.6 BIOLOGICAL ASPECTS IN HUMANS

A.6.1 METABOLISM

A.6.1.1 Uptake and Absorption

Uptake and absorption data for chlorophenols is derived mainly from experimental animals. Documented human systemic toxicity cases are only available for pentachlorophenol. Absorption can occur via the oral, cutaneous, or respiratory routes in humans. Information on absorption mechanisms and rates for lower chlorophenols is not available. Table A.6.1 summarizes information on the absorption of chlorophenols derived primarily from toxicity data. Chlorophenol toxicity in experimental animals has been demonstrated following parenteral injection with appropriately high doses; oral toxicity has also been demonstrated. Respiratory absorption has been shown only for pentachlorophenol. The effects of cutaneous absorption depend on the degree of chlorination of the phenol. 2-Chlorophenol is readily absorbed through the skin, but 2,4-dichlorophenol is absorbed less rapidly. The 2,4,5- and 2,4,6-trichlorophenol isomers are not absorbed by the cutaneous route, at least in toxic amounts, but 2,3,4,6-tetrachlorophenol and pentachlorophenol are absorbed readily.

Some information on the uptake of pentachlorophenol by humans and experimental animals is available. Systemic toxicity can result from cutaneous or oral exposure to the compound. Some differences in the relative toxicities of pentachlorophenol and sodium pentachlorophenate are evident. Pentachlorophenol administered by the oral or cutaneous route appears to be approximately twice as toxic as sodium pentachlorophenate. The toxicity of pentachlorophenol depends on the solvent in which it is carried, but no explanation has been provided for this phenomenon. It seems likely that the absorption and/or toxicity of other chlorophenols may depend on the solvent in which the compounds are dissolved prior to contact, but more information is needed.

A.6.1.2 Transport and Distribution

No information is available on the transport and distribution of lower chlorophenols in humans and experimental animals. However, limited information is available on the tissue distribution of some of the trichlorophenol and tetrachlorophenol isomers in experimental animals following administration of organochlorine precursor compounds which yield chlorophenols as metabolites. The data are difficult to interpret because distribution sites within the organism may depend on factors such as the sites within the body where the chlorophenol is produced and perhaps the site within the body where the precursor preferentially locates.

Sheep fed a diet containing the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) had residues of 2,4,5-trichlorophenol in liver, kidney, and muscle tissues but not in fat. When the diets containing 2,4,5-T were withdrawn one week prior to slaughter, 2,4,5-trichlorophenol

TABLE A.6.1. ABSORPTION OF CHLOROPHENOLS BY HUMANS AND EXPERIMENTAL ANIMALS

Compound	Route of administration					
	Oral	Cutaneous	Respiratory	Subcutaneous	Intraperitoneal	Intravenous
2-Chlorophenol	Yes	Yes		Yes	Yes	Yes
2,4-Dichlorophenol	Yes	Probably		Yes	Yes	
2,4,5-Trichlorophenol	Yes	No		Yes	Yes	
2,4,6-Trichlorophenol	Yes	No			Yes	
2,3,4,6-Tetrachlorophenol	Yes	Yes		Yes	Yes	
2,3,5,6-Tetrachlorophenol					Yes	
Pentachlorophenol	Yes[a]	Yes[a]	Yes[a]	Yes	Yes	Yes

[a] Determined from human data.

levels in muscle decreased, but liver and kidney tissues retained most of the accumulated 2,4,5-trichlorophenol. When rats were dosed with lindane for 19 consecutive days, 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol were found in the heart. The liver contained detectable levels of 2,3,4,6- and 2,3,5,6-tetrachlorophenol, and the kidneys contained 2,4,6-trichlorophenol and both tetrachlorophenols. Available data indicate that urine is the primary route of elimination. In view of the known detoxification role of the liver, it is not surprising to find chlorophenol residues in this organ. If extrapolation from pentachlorophenol data is valid, chlorophenols are likely transported in the blood. Whether pentachlorophenol is absorbed through the skin, via the respiratory tract, or through injection, it is distributed by the blood. Substantial data have shown the presence of pentachlorophenol in the blood following uptake by each of these routes. In addition, autopsy reports on victims poisoned by pentachlorophenol show the presence of pentachlorophenol in the blood. Pentachlorophenol apparently does not bind to blood cells, but evidence suggests binding to plasma protein. This binding may account for the slower rate of pentachlorophenol loss in the later stages of excretion.

Data for tissue distribution following uptake of pentachlorophenol by humans is derived mainly from autopsy results of fatal cases of pentachlorophenol intoxication. These cases have consistently revealed the presence of the compound in the liver, kidney, and usually the stomach. Detectable residues also are occasionally seen in the brain, adrenal glands, heart, lungs, and connective tissue. A recent survey indicated that parts-per-billion levels of pentachlorophenol were present in adipose tissue samples taken from the general population. The definitive kinetics of pentachlorophenol accumulation in fat are unknown and are worthy of further investigation.

The postmortem findings in humans poisoned by pentachlorophenol are similar to findings in experimental animals. Pentachlorophenol appears in most tissues (including stomach, intestines, kidney, liver, and gallbladder) of rabbits, mice, and rats dosed by the cutaneous, oral, or intraperitoneal routes. A scheme for pentachlorophenol transport and distribution in mammals is presented in Section D.6.1.2. Transfer of pentachlorophenol across the placenta requires more study.

A.6.1.3 Biotransformation

Metabolic alteration of chlorophenols in mammals has been studied in experimental animals, and data on the metabolic alterations of pentachlorophenol in humans have recently been reported. Very little information on the metabolism of lower chlorophenols following uptake by mammals is available. The metabolic fate of the chlorophenols formed from other organochlorine compounds in the body may reflect the fate of chlorophenols administered directly to animals. Data on chlorophenol metabolism in higher animals indicate that conjugation is a major metabolic route. Conjugation to sulfuric and glucuronic acids has been demonstrated for chlorophenols following direct administration and following the production of chlorophenols from precursor compounds.

2-Chlorophenol was excreted in the urine of dogs in free and conjugated forms. Following administration, 87% of the compound was excreted in the form of sulfuric and glucuronic acid conjugates. Free 2-chlorophenol as well as conjugates of sulfuric and glucuronic acids have been detected in rabbits given chlorobenzene. Some investigators have suggested that ortho methylation may be another route for 2-chlorophenol detoxification in mammals, but data are not available. 2,4-Dichlorophenol was produced in mice following administration of β- or γ-hexachlorocyclohexane. 2,4-Dichlorophenol was excreted in the free state as well as in the form of sulfate and glucuronide conjugates; other metabolites included 2,4,6-trichlorophenol and its sulfate and glucuronide conjugates and trace amounts of 2,4,5-trichlorophenol and its conjugates. Rats excreted 2,4,5-trichlorophenol and 2,4,5-trichlorophenol conjugates after administration of 4-(2,4,5-trichlorophenoxy)-butyric acid. The 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol isomers and their glucuronide conjugates were detected in urine from rats dosed with pentachlorophenol. Data on other metabolic pathways for lower chlorophenols are not available, although conjugation is a major metabolic fate of these compounds in some experimental animals.

More data are available on the metabolic fate of pentachlorophenol in experimental mammals. Conjugate formation occurs frequently with pentachlorophenol. The formation of pentachlorphenyl-β-glucuronide has been reported in rabbits. The production of tetrachlorohydroquinone and its conjugates has been verified in mice and rats following pentachlorophenol administration. Furthermore, chloranil, a compound closely related to tetrachlorohydroquinone, has been reported as a constituent of rabbit urine following administration of pentachlorophenol. A proposed metabolic pathway for pentachlorophenol in mammals has been constructed based on available data (Section D.6.1.3). Because urinary levels of pentachlorophenol have been used to estimate pentachlorophenol exposure in humans, a true assessment of the degree of exposure should include analysis of urine for tetrachlorohydroquinone, chloranil, and the conjugated compounds.

Large amounts of pentachlorophenol are found in the liver relative to other tissues. Based on the known role of the liver in detoxification of foreign substances, it is probable that metabolic breakdown takes place in the liver, but direct information is not currently available. Detoxification of other chlorophenols may also occur in this organ.

A.6.1.4 Elimination

Following human exposure to chlorophenols, the primary mode of elimination has been shown to be through the urine. Information on the elimination of chlorophenols other than pentachlorophenol is sketchy.

Following administration of 2-chlorophenol to dogs, 87% of the compound was excreted in conjugation with sulfuric and glucuronic acids in the urine. 2-Chlorophenol results from metabolism of chlorobenzene in rabbits, and the compound is excreted in free and conjugated forms in the urine; excretion rates are not available. 2,4-Dichlorophenol has been detected in rabbit and rat urine following nemacide [*O*-(2,4-dichlorophenyl)*O*,*O*-diethyl phosphorothioate] administration. 2,4-Dichlorophenol is apparently the major metabolite of nemacide in mammals. Approximately 70% of the nemacide dose appeared in the urine as 2,4-dichlorophenol within three days of administration; the fate of the remaining 30% of the compound is not known.

Following injection of the β- or γ-hexachlorocyclohexane isomers into mice, a number of chlorophenols and their conjugates, including 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,5-tetrachlorophenol, appeared in the urine. When pentachlorophenol was administered to rats, 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol were detected as metabolites in the urine.

Present evidence indicates that the lindane metabolites 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,5- and 2,3,4,6-tetrachlorophenol are excreted by rats for at least one month following treatment; this may reflect the rate of lindane metabolism. On the other hand, following administration of ronnel [*O*,*O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate], rats excreted 2,4,5-trichlorophenol for two days. The difference in these rates likely depends on the chemical nature of the precursor compound. Although lack of information precludes definitive conclusions, it is speculated that the tetrachlorophenol compounds in particular may behave in a manner similar to pentachlorophenol because their chemical structures and lipid solubilities are similar. Lower chlorophenols should be excreted more rapidly due to their higher water solubility.

Although the primary mode of pentachlorophenol excretion appears to be the urine, fecal excretion also plays a role. Values ranging from 4% of the injected dose in the rabbit to 14% in rats over a ten-day period have been reported in the feces. In humans, initial elimination of pentachlorophenol in the urine may be very rapid, but return to urinary background levels of pentachlorophenol may take as long as one month. It is difficult to determine from the studies whether all of the pentachlorophenol has been excreted within this time, whether some may be stored in various deposits in the body, or whether there was continuous exposure. A two-component urinary excretion pattern of pentachlorophenol by rats has been demonstrated.

Dose and route of exposure also apparently affect the excretion rate of pentachlorophenol. In humans, pentachlorophenol is excreted more rapidly at high dose levels than when the body burden is lower. The exact relationships between excretion rate and dose and between excretion rate and route of exposure have not been verified. In mammals, the bulk of pentachlorophenol administered appears to be excreted relatively rapidly — approximately 50% is excreted in the urine in 24 hr for rats, rabbits, and mice and 70% to 85% is eliminated in four days. Excretion of the remaining pentachlorophenol may take longer, and the effect of this low-level contamination on the organism is unknown.

The applicability of the information on pentachlorophenol elimination to other chlorophenols is uncertain. However, elimination of at least the tetrachlorophenols may parallel that of pentachlorophenol.

A.6.2 EFFECTS

A.6.2.1 Physiological or Biochemical Role

There is no evidence that chlorophenols play a normal biochemical or physiological role in humans or mammals.

A.6.2.2 Toxicity

Except for pentachlorophenol, most information on the general toxicity of chlorophenols to humans is derived from investigations with experimental animals. Documented human toxicity following pentachlorophenol exposure is available, and much of the information on symptoms and pathological changes is derived from these cases. No documented reports of human systemic toxicity due to exposure to lower chlorophenols are available.

A.6.2.2.1 Mechanism of Action — All chlorophenol isomers examined in this report appear to uncouple oxidative phosphorylation in higher mammals and humans, leading to serious metabolic disturbances. The potency of these compounds in producing this effect appears to increase with increased chlorination of the parent phenol. The effect of chlorophenols on oxidative phosphorylation in rat liver mitochondrial preparations (Table A.6.2) and in rat brain homogenates has been determined. Pentachlorophenol is the strongest inhibitor of oxidative phosphorylation, followed by 2,3,4,6-tetrachlorophenol; tetrachlorophenol possesses about 50% of the activity seen with pentachlorophenol. Decreasing chlorination results in decreased activity; 2,4,5-trichlorophenol possesses approximately 30% and 2,4,6-trichlorophenol possesses 5% of the activity of pentachlorophenol. 2-Chlorophenol was the weakest chlorophenol tested in this in vitro assay system.

Typical clinical signs of pentachlorophenol poisoning in experimental animals correspond closely with clinical symptoms from poisoning by higher chlorophenol isomers. Present evidence suggests that the toxic action of chlorophenols, with the possible exception of 2-chlorophenol, in experimental animals is due to inhibition of oxidative phosphorylation, thereby short-circuiting metabolism. The dosages or concentrations required to elicit this effect are different for different compounds, but with sufficient uptake of any of these compounds, symptoms similar to the toxicological picture found with pentachlorophenol are likely to ensue.

The mechanism of action of 2-chlorophenol may be different. 2-Chlorophenol is capable of causing convulsions in experimental animals. Phenol can cause convulsions with no apparent effect on oxidative phosphorylation. 2-Chlorophenol is apparently more effective in eliciting convulsions than phenol. Coupled with its relatively weak action on the energy systems of the cell, this evidence indicates that the mechanism of

TABLE A.6.2. EFFECT OF CHLOROPHENOLS ON OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

Compound	I_{50}[a] (micromoles/liter)	I_{50}[a] (mg/liter)
2-Chlorophenol	520	67
2,4-Dichlorophenol	42	7.0
2,4,6-Trichlorophenol	18	3.6
2,4,5-Trichlorophenol	3	0.59
2,3,4,6-Tetrachlorophenol	2	0.46
Pentachlorophenol	1	0.27

[a] Concentration required to decrease oxidative phosphorylation to 50% of normal levels.

Source: Adapted from Mitsuda, Murakami, and Kawai, 1963, Table I, p. 369. Reprinted by permission of the publisher.

action of 2-chlorophenol may more closely resemble that found in phenol poisoning; however, more information is needed. A convulsive effect is also noted when 2,4,6-trichlorophenol is administered to experimental animals. As Table A.6.2 indicates, the effect of 2,4,6-trichlorophenol on oxidative phosphorylation is weaker than that of other higher chlorophenols. Thus, in some respects, 2,4,6-trichlorophenol poisoning may have a mechanism of action similar to that of 2-chlorophenol in experimental animals.

A general property of uncouplers is the ability to inhibit the enzymes lactate dehydrogenase and hexokinase in in vitro systems. 2,4-Dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol are all capable of inhibiting these enzymes; 2-chlorophenol has not been tested.

Pentachlorophenol is clearly a strong uncoupler of oxidative phosphorylation, but the molecular mechanism remains unclear. Biochemical effects of pentachlorophenol have been divided into three groups, depending on the pentachlorophenol concentration. Low concentrations uncouple oxidative phosphorylation and result in an increased respiration. High levels drastically curtail glycolytic phosphorylation, causing inactivation of respiratory enzymes with a drastic reduction in respiration. Intermediate amounts inhibit the action of mitochondrial ATPase and myosin ATPase. Convincing evidence exists that binding of pentachlorophenol to mitochondrial protein is necessary for any of these biochemical processes to occur. The basic effect of pentachlorophenol in uncoupling oxidative phosphorylation may be responsible for all effects at the biochemical level. The effects noted with high pentachlorophenol levels may be due to (1) the drastic curtailment of metabolic processes in the cell and subsequent breakdown in other enzyme systems or (2) the direct binding of pentachlorophenol to other crucial enzymes in the cell and inactivation of other essential pathways. Total breakdown of mitochondrial integrity has been reported and could be the final stage of pentachlorophenol toxicity in the cell.

A.6.2.2.2 Local and Systemic Pathology — All chlorophenols tested are irritating to the nose, throat, skin, and eyes. Local effects of the various chlorophenols are summarized in Table A.6.3; detailed information is found in later sections of the report. Chloracne has occurred in humans following prolonged (over a period of years) contact with several chlorophenol isomers (Table A.6.3). It is likely that chloracne largely results from exposure to impurities found commonly in commercial chlorophenol preparations. These impurities, namely chlorodibenzo-*p*-dioxins, are capable of causing chloracne when they are applied in the pure state.

TABLE A.6.3. LOCAL EFFECTS OF CHLOROPHENOLS ON HUMANS OR EXPERIMENTAL ANIMALS

Compound	Skin effects			Eye effects	
	Redness and edema	Chloracne	Chemical burn	Irritation	Corneal injury
2-Chlorophenol	Yes		Yes	Yes	Yes
2,4,-Dichlorophenol		Maybe			
2,4,5-Trichlorophenol	Yes	Yes	Maybe	Yes	Yes
2,4,6-Trichlorophenol	Yes		Maybe	Yes	Yes
2,3,4,6-Tetrachlorophenol				Yes	Yes
2,3,5,6-Tetrachlorophenol				Yes	Yes
Tetrachlorophenol[a]		Yes		Yes	Yes
Pentachlorophenol	Yes	Yes	Yes	Yes	Yes

[a] Isomer not identified.

Except for pentachlorophenol, information on the tissue pathology of experimental animals or humans following chlorophenol intoxication is scant. Administration of 2-chlorophenol to the rat or the blue fox resulted in marked kidney injury, fatty infiltration of liver tissue, renal granular dystrophy, and necrosis of the stomach and intestinal mucosa. Chronic feeding of 2,4-dichlorophenol to mice at daily levels up to 230 mg/kg for six months resulted in only minor histological changes in the liver. 2,4-Dichlorophenol is considered a relatively safe substance, with 100 mg/kg daily as a maximum no-effect level. Similarly, 2,4,5-trichlorophenol appears to be only mildly toxic. Rabbits fed 20 doses of the compound (500 mg/kg in each dose) showed mild, reversible kidney and liver changes. Rats fed 2,4,6-trichlorophenol at levels of 10 to 1000 mg/kg body weight showed no adverse effects at levels less than 100 mg/kg daily as judged by gross appearance and behavior, mortality, food consumption, growth, hematologic values, final average body and organ weight ratios, or gross and microscopic examination of the tissues. At 300 or 1000 mg/kg daily, rats sustained minor microscopic damage to the kidneys and liver. The pathologist stated that the changes in the liver and kidneys of the animals were mild, reversible, and probably of minor significance. Pentachlorophenol data from experimental animal studies and autopsies of fatally poisoned humans have been reported. Autopsies of victims showed moderate to severe pathological changes in the gastrointestinal tract, lungs, spleen, liver, kidneys, and cardiovascular system. The pathological changes of pentachlorophenol intoxication are discussed in Section D.6.2. The general hyperpyrexia caused by pentachlorophenol may be the indirect cause of many of these pathological changes.

A.6.2.2.3 Acute Toxicity — The exact dosage in accidental or suicidal poisoning cases is difficult to determine; therefore, experimental animal studies are preferable for comparing the toxic effects of chlorophenols. Some typical LD_{50} values for chlorophenols are compiled in Table A.6.4. As shown in the table, pentachlorophenol is the most toxic phenol, although the toxicity of 2,3,4,6-tetrachlorophenol is close to that of pentachlorophenol. 2-Chlorophenol appears to be approximately five times less toxic than higher chlorophenols; 2,4-dichlorophenol is still less toxic, and the trichlorophenol isomers must be administered in gram-per-kilogram quantities to cause death in experimental animals.

Chlorophenols are separated into two general categories on the basis of their toxicological effects: (1) the convulsive chlorophenols, including 2-chlorophenol and 2,4,6-trichlorophenol, and (2) the nonconvulsive chlorophenols. Convulsions and tremors are very prominent when experimental animals are treated with convulsive phenols; pronounced hypotonia and coma are characteristic of poisoning by the nonconvulsive chlorophenols. Poisoning by all chlorophenols is characterized by a marked rise in temperature and, in most cases, an initial increase in respiratory rate followed by a decreased rate and onset of coma. Clinical signs of poisoning by the convulsive phenols generally occur in the following sequence: (1) soon after administration of the compound, restlessness and an increased rate of respiration are evident; (2) somewhat later motor weakness develops and tremors and convulsions are induced by noise or touch; (3) eventually, dyspnea, coma, and death occur. The clinical symptoms of poisoning with nonconvulsive chlorophenols is quite different. All of these symptoms apply to poisoning by pentachlorophenol. Toxicological symptoms of poisoning by other nonconvulsive chlorophenols resemble those for pentachlorophenol, but all of these symptoms may not be present when mammals are exposed to some of the lower chlorinated phenols. Tetrachlorophenol poisoning appears to most closely resemble pentachlorophenol poisoning. Symptoms in humans poisoned by acute or chronic high-level doses of pentachlorophenol include weight loss, general weakness, fatigue, dizziness, mental weakness, headache,

TABLE A.6.4. ACUTE TOXICITY OF CHLOROPHENOLS TO EXPERIMENTAL ANIMALS

Compound	Animal	Route of administration	LD_{50} (mg/kg)	Source
2-Chlorophenol	Rat	Oral	670	Christensen and Luginbyhl, 1975
	Rat	Subcutaneous	950	Christensen and Luginbyhl, 1975
	Albino rat	Intraperitoneal	230	Farquharson, Gage, and Northover, 1958
	Rabbit	Subcutaneous	950[a]	Christensen and Luginbyhl, 1975
	Rabbit	Intraveneous	120[a]	von Oettingen, 1949
	Guinea pig	Subcutaneous	800[a]	Christensen and Luginbyhl, 1975
	Mouse	Oral	670	Bubnov, Yaphizov, and Ogryzkov, 1969
	Blue fox	Oral	440	Bubnov, Yaphizov, and Ogryskov, 1969
	Unknown mammal	Oral	440	Christensen and Luginbyhl, 1975
2,4-Dichlorophenol	Rat	Oral	580	Christensen and Luginbyhl, 1975
	Rat	Intraperitoneal	430	Christensen and Luginbyhl, 1975
	Rat	Subcutaneous	1730	Christensen and Luginbyhl, 1975
	Mouse	Oral	1600	Christensen and Luginbyhl, 1975
2,4,5-Trichlorophenol	Rat	Oral	2830	Howard and Durkin, 1973
	Rat	Oral	2460	Howard and Durkin, 1973
	Rat	Oral	820	Deichmann, 1943
	Rat	Oral	2960	McCollister, Lockwood, and Rowe, 1961
	Rat	Subcutaneous	2260	Deichmann, 1943
	Rat	Intraperitoneal	355	Farquharson, Gage, and Northover, 1958
	Unknown mammal	Unknown	150[a]	Christensen and Luginbyhl, 1975
	Rat	Oral	1620[b]	Howard and Durkin, 1973
2,4,6-Trichlorophenol	Rat	Oral	2800	Gosselin et al., 1976
	Rat	Oral	820	Christensen and Luginbyhl, 1975
	Rat	Intraperitoneal	276	Farquharson, Gage, and Northover, 1958
2,3,4,6-Tetrachlorophenol	Rat	Oral	140	Deichmann, 1943
	Rat	Subcutaneous	210	Deichmann, 1943
	Mouse	Subcutaneous	120	Bechold and Ehlrich, 1906
	Rat	Intraperitoneal	130	Farquharson, Gage, and Northover, 1958
	Mouse	Intraperitoneal	250[a]	Christensen and Luginbyhl, 1975
2,3,5,6-Tetrachlorophenol	Mouse	Intraperitoneal	500[a]	Christensen and Luginbyhl, 1975
Pentachlorophenol	Rat	Oral	78	Deichmann et al., 1942
	Rabbit	Oral	70–130	Flickinger, 1971
	Guinea pig	Oral	50–140	Flickinger, 1971
	Rabbit	Cutaneous	100–200	Dow Chemical Company, 1969*a*
	Rabbit	Subcutaneous	70–85	Deichmann et al., 1942
Sodium pentachlorophenate	Rat	Oral	210	Dow Chemical Company, 1969*b*
	Rabbit	Oral	275	Dow Chemical Company, 1969*b*
	Guinea pig	Oral	80–160	Dow Chemical Company, 1969*b*
	Rabbit	Cutaneous	100–300	Dow Chemical Company, 1969*b*

[a] Minimum lethal dose.

[b] Sodium salt of 2,4,5-trichlorophenol.

anorexia, nausea and vomiting, dyspnea, hyperpyrexia, respiratory distress, tachycardia, hepatomegaly, profuse perspiration, and elevated basal metabolic rate. The most common symptoms of poisoning are general weakness, weight loss, and profuse perspiration. Fever and profuse sweating result from the uncoupling of oxidative phosphorylation. Hyperpyrexia may be the primary factor leading to circulatory and respiratory failure due to the central and peripheral effects of fever as in heat stroke. Although the exact dose of pentachlorophenol causing illness in humans is not known, data indicate that pentachlorophenol quantities absorbed through the skin of newborn infants can be lethal.

Fifty-one cases of poisoning from pentachlorophenol ingestion or absorption have been reported, with 30 resulting in death. No specific antidote is known, and death can occur in spite of conventional supportive therapy. In a recent case, the use of intravenous fluids and electrolytes was successful. In acute pentachlorophenol poisoning, onset of symptoms is very rapid and death occurs in 2 to 3 hr. In nonfatal cases of pentachlorophenol poisoning, onset of symptoms occurs at blood concentrations of 40 to 80 mg/liter with corresponding concentrations of 40 to 80 mg/liter in urine. Blood levels in fatal cases ranged from 46 to 156 mg/liter and urine levels ranged from 28 to 520 mg/liter. The acute oral lethal dose of pentachlorophenol has been listed as 29 mg/kg body weight for humans; however, the actual amount required to cause systemic toxicity in humans is unknown and probably depends on the renal competency of the individual. For individuals whose ability to excrete the compound is reduced, the lethal dose may be considerably lower. Most cases of fatal and nonfatal human pentachlorophenol intoxication have resulted from improper handling of the compound. Elevated pentachlorophenol levels have been reported in the blood and urine of workers in the wood preservation industry.

A.6.2.2.4 Chronic Toxicity

A.6.2.2.4.1 In experimental animals — The chronic toxicity of lower chlorophenols in experimental animals has not been adequately studied. Mice fed 2,4-dichlorophenol over a six-month period were evaluated for average body weight, food consumption, organ weight, and histology of major organs. No adverse changes in the behavior, growth rate, or levels of blood glutamic oxaloacetate and glutamic or pyruvate transaminases were found in mice receiving up to 230 mg/kg daily; minor histological changes occurred in the liver. A similar low chronic toxicity has been demonstrated for 2,4,5-trichlorophenol. Rats daily fed 2,4,5-trichlorophenol at levels up to 1000 mg/kg body weight sustained only minor microscopic damage to the kidneys and liver. Moderate, probably reversible, changes occurred in animals receiving 2,4,5-trichlorophenol at daily dosages greater than 300 mg/kg body weight. It has been speculated that the toxicity of 2,4,6-trichlorophenol and 2-chlorophenol may parallel the relatively low toxicity of 2,4-dichlorophenol or 2,4,5-trichlorophenol. Chronic effects of the tetrachlorophenol isomers, on the other hand, are speculated to more closely resemble the chronic effects documented for pentachlorophenol. Specific information is not available.

No chronic systemic disease was noted in rabbits when pentachlorophenol was applied cutaneously at levels low enough to avoid gross skin damage. Daily subcutaneous or intraperitoneal doses of pentachlorophenol to rabbits may cause severe symptoms and death if the doses are high enough. The amount of pentachlorophenol in small divided doses necessary to cause death is very similar to the minimum lethal dose with a single administration of the compound. Renal competency may determine the chronic levels of pentachlorophenol which may be tolerated. Chronic poisoning results not from a storage-type accumulation of the compound in the system, but rather from uptake of the substance in excess of its excretion rate. Rats fed diets containing pentachlorophenol at 1 to 3 mg/kg for 90 days to two years did not show adverse health effects. Levels of 10 to 30 mg/kg caused some changes but were not lethal.

In summary, lower chlorophenols appear to be only mildly toxic following high-level chronic doses. Chronic pentachlorophenol poisoning can result when daily exposures are sufficient to achieve a steady-state level of pentachlorophenol in the organism which is high enough to cause systemic intoxication. Chronic effects resulting from storage-type accumulation of pentachlorophenol or any other chlorophenols in experimental animals have not been demonstrated and are not expected to occur because of the rapid elimination of chlorophenols.

A.6.2.2.4.2 In humans — Chronic effects resulting from low-dose exposure to chlorophenols have not been reported. Long- or short-term chronic effects following exposure to chlorophenols other than pentachlorophenol have not been demonstrated. Apparently, low-dose pentachlorophenol exposure does not result in chronic effects in humans. Although low, but detectable, levels of pentachlorophenol have been found in the blood and urine of nonoccupationally exposed persons, chronic effects resulting from these prevalent levels have not been demonstrated. Chronic exposure to pentachlorophenol among individuals involved in the wood-treatment industry may have reversible effects on kidney function. Significant differences in blood and urinary phosphorus levels and in creatinine clearance have been found in wood treaters before, during, and after vacation. It was concluded that pentachlorophenol exposure results in reversible decreases in creatinine clearance and phosphorus reabsorption in the kidney. Such minor effects on kidney function are probably insignificant in most cases; however, the question warrants further study.

Other evidence indicates that workers chronically exposed to pentachlorophenol may have significantly higher prevalence of C-reactive protein in the sera. Although the clinical significance of these elevated levels is not known, C-reactive protein levels are often elevated in acute states of various inflammatory disorders or tissue damage. It has been inferred that the elevated levels of C-reactive protein in individuals exposed to pentachlorophenol indicate inflammation or tissue injury. These data have not been verified, and the significance of elevated levels of C-reactive protein in individuals exposed to pentachlorophenol remains speculative.

Pentachlorophenol levels of 12 to 45 μg/kg have been found in fat of individuals not occupationally exposed. The significance of these pentachlorophenol levels in the fat is unknown; pentachlorophenol in fat at these levels is toxicologically insignificant. Levels of pentachlorophenol in the fat of individuals actively exposed to the compound in their daily work have not been reported. The potential for pentachlorophenol biomagnification in human adipose tissue is an open question warranting further investigation.

The nearly ubiquitous presence of pentachlorophenol in the human population may result in part from the metabolism of other organochlorine compounds. For example, pentachlorophenol has been isolated from the excreta of rats and monkeys following administration of lindane. These results suggest an alternative explanation for the nearly ubiquitous presence of pentachlorophenol in the human population and question the tacit assumption that pentachlorophenol in human urine, serum, and tissue results from exposure to pentachlorophenol. Additional investigations in these areas are urgently needed.

A.6.2.3 Carcinogenicity

Pentachlorophenol and 2,4,6-trichlorophenol have shown no significant indication of tumorigenicity following oral administration to mice. However, repeated application of phenol and some substituted phenols is reportedly capable of promoting the appearance of skin tumors in mice following a single initiating dose of dimethylbenzanthracene. Tumors also developed in mice (not exposed to dimethylbenzanthracene) treated with phenol alone over a long period. When 2-chlorophenol, 2,4-dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol, and pentachlorophenol were tested in a similar system, no tumorigenic or tumor-promoting activity was seen with 2,4,6-trichlorophenol or pentachlorophenol; 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol possessed approximately the same activity in promoting tumor formation as phenol. Both benign and malignant tumors were formed. However, no tumorigenic effect was seen in the absence of an initiating dose of dimethylbenzanthracene.

A.6.2.4 Teratogenicity

2,4,5-Trichlorophenol and pentachlorophenol are the only chlorophenols tested for possible teratogenic effects in experimental animals. No teratogenic effect of 2,4,5-trichlorophenol was seen at daily doses of 0.9 or 9 mg/kg body weight administered on days 6 through 15 of pregnancy. Pentachlorophenol is fetotoxic but is not truly teratogenic in rats and the golden Syrian hamster.

A.6.2.5 Mutagenicity

Data on possible mutagenic effects of lower chlorophenols are not available. Pentachlorophenol did not demonstrate mutagenic properties when tested in a microbiological or mammalian system or when tested for mutagenic properties in the fruit fly (*Drosophila melanogaster*).

A.6.2.6 Aplastic Anemia

One case of aplastic anemia due to pentachlorophenol and a tetrachlorophenol isomer has been reported. The individual was exposed to a formulation containing pentachlorophenol and tetrachlorophenol. Because the postmortem examination failed to reveal any other cause for aplastic anemia, it was concluded that the damage to the bone marrow was caused by these chlorophenols. No reports of aplastic anemia resulting from exposure to other chlorophenols were found.

SECTION A.6

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A.7 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

A.7.1 TRENDS IN PRODUCTION AND USE

Among the chlorophenols examined in this report, only three — 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol — have been used to any large extent in the environment. 2,4,6-Trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol are mainly used as fungicides and microbicides, particularly for the preservation of wood, leather, and glue. 2,4,6-Trichlorophenol and 2,3,4,6-tetrachlorophenol are used to only a small extent, but pentachlorophenol is widely used in the wood preservation industry as well as for preservation of a wide variety of other materials. Pentachlorophenol has also been used as a herbicide and preharvest desiccant, as an algicide or molluscicide, and in food processing plants and pulp mills for the control of slime and mold. Although production figures for 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol are not available, the production of pentachlorophenol (26,000,000 kg in 1976) far exceeds the estimated production and usage of the other two chlorophenols. Obviously, the potential for environmental contamination is greatest for pentachlorophenol.

2-Chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol are not used to any large extent as preservatives or microbicides. The principal uses of these compounds are as intermediates for the manufacture of higher chlorophenols or as intermediates for the manufacture of other organic compounds, including the widely used herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Other phenoxyalkanoic herbicides are also manufactured with these compounds as precursors. Because the direct usage of these compounds as bactericides or germicides is probably negligible, environmental contamination is most likely to result from degradation of commonly used pesticides in the environment.

A.7.2 SOURCES OF POLLUTION

Information on the atmospheric distribution of chlorophenols other than pentachlorophenol is not available. Discharges of lower chlorophenols to the atmosphere may occur in facilities where these compounds and the phenoxyalkanoic herbicides are manufactured. Because all lower chlorophenols are relatively volatile, volatilization from water, soil, foliage, and impervious surfaces may play an important role in their dispersal to the atmosphere. Incineration of containers and trash containing chlorophenols could generate volatile products to the atmosphere.

Monitoring data for pentachlorophenol in the general atmosphere are unavailable. Circumstantial evidence indicates that pentachlorophenol may be present in the atmosphere. Levels of pentachlorophenol in rainwater and snow samples taken from the Mauna Kea Summit in Hawaii indicate that pentachlorophenol is likely present in the atmosphere either as a vapor or as an occlusion of dust particles and that the compound is likely removed from the atmosphere by washout. Pentachlorophenol may be discharged in gaseous and particulate forms from chemical firms that manufacture pentachlorophenol, but detailed information on pentachlorophenol levels in the atmosphere surrounding such plants is not available.

Monitoring data on the presence of monochlorophenols, dichlorophenols, trichlorophenols, and tetrachlorophenols in air, water, or soil are not available. Monitoring data for pentachlorophenol levels in water are also rare. Fish kills following industrial accidents involving spillage and seepage of concentrated pentachlorophenol as well as deleterious effects on aquatic organisms following the widespread distribution of pentachlorophenol as a molluscicide or algicide have been reported. However, these lethal occurrences are usually associated with the presence of extremely high levels of pentachlorophenol in water. Documentation of low-level pentachlorophenol contamination of water is not available. Pentachlorophenol is known to be present in treated and untreated wastewaters from wood treatment plants. Pentachlorophenol has also been detected in storm water draining industrial sites where treated lumber has been stored and in municipal sewage. Measurable pentachlorophenol levels have been detected in the Willamette River in Oregon, in a creek in Pennsylvania, and in the drinking water supplies of Tallahassee, Florida, and Corvallis, Oregon. Levels are generally low, and the presence or absence of pentachlorophenol residues in other water bodies is speculative.

Industrial waste discharge is likely the principal point source of water pollution by chlorophenols. During the manufacture of chlorophenols and the widely used herbicides 2,4-D, 2,4,5-T, and silvex, chemical waste is generated from incomplete reaction of the starting material, by-product formation, and incomplete recovery of desired products. These wastes contain a variety of phenols and other compounds. A typical waste arising from the manufacture of 2,4-D and 2,4,5-T contains 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and other chlorinated phenols. Runoff from urban and agricultural watersheds could be an important nonpoint source of chlorophenols in aquatic environments, but this has not been reported. Primary sources include the presence of chlorophenols as impurities in commercial formulations of the phenoxyalkanoic herbicides and as degradation products of these compounds in soils.

The primary source of soil contamination by chlorophenols is probably through the application of the phenoxyalkanoic herbicides 2,4-D, 2,4,5-T and its derivatives, and silvex. Additionally, trichlorophenols and tetrachlorophenols may appear in soil folllowing application and degradation of pentachlorophenol or lindane. Soil contamination by pentachlorophenol is likely indirect except when the chemical is used as a herbicide or preharvest desiccant. Monitoring data for chlorophenol levels in soil are not available, except for soil migration studies around pentachlorophenol-treated poles.

A.7.3 ENVIRONMENTAL FATE

A.7.3.1 Mobility and Persistence in Air

The movement, fate, and persistence of chlorophenols in the atmosphere are matters for speculation. Circumstantial evidence (the detection of pentachlorophenol in rainwater and snow) indicates that pentachlorophenol is present in the atmosphere; however, the presence of any other chlorophenol in the atmosphere has not been documented.

A.7.3.2 Mobility and Persistence in Aquatic Environments

Chlorophenols may be present in aquatic environments in one or more of the following three forms: (1) dissolved in either free or complexed form, (2) adsorbed to suspended and/or bottom sediments, or (3) carried in biological tissues. Movement of the compounds in water has not been well studied, but it is believed that mobility depends primarily on hydrological considerations such as currents and, in the case of transportation by organisms, on movement and migration of the organisms. The persistence of chlorophenols in the aquatic environment depends on a number of environmental variables, but the interrelationships have not been fully characterized. Dissipation of chlorophenols from the aqueous phase may occur by volatilization to the atmosphere, photoinactivation, sorption to suspended and/or bottom sediments, or biodegradation. The contribution of volatilization to the dissipation of chlorophenols from aquatic systems is unclear. The pentachlorophenol content of aerated solutions decreases rapidly in laboratory experiments, presumably due to volatilization. The higher vapor pressures of other chlorophenols suggest that volatilization plays a role in their dissipation from water; however, definitive data are not available.

Many organic compounds, including chlorophenols, undergo photochemical decomposition when exposed to ultraviolet light. Photochemically induced degradation occurs at surfaces of airborne particulates and water. In finding their way to surfaces by various mechanisms, chlorophenols are exposed to sunlight and are thereby susceptible to photochemical degradation. Information on photodecomposition of 2-chlorophenol is not available. 2,4-Dichlorophenol rapidly degrades in aqueous solutions exposed to ultraviolet light. Direct information on the photochemical reactions of trichlorophenols and tetrachlorophenols is scant. Insight into their photodecomposition has been obtained from closely related compounds such as pentachlorophenol and 2,4,5-T. 2,4,5-Trichlorophenol is subject to rapid photodecomposition. Information is lacking on the photodecomposition of 2,4,6-trichlorophenol or any of the tetrachlorophenol isomers. These compounds are likely subject to photodecomposition when conditions are appropriate (i.e., water of sufficient clarity to allow adequate ultraviolet penetration). Evidence exists that pentachlorophenol and sodium pentachlorophenate are subject to rapid photodecomposition in aqueous solution. Practical experience in the field indicates that photodecomposition may be a primary mode of pentachlorophenol dissipation in aquatic systems under some conditions. The extent of photoinduced losses of chlorophenols in natural systems has yet to be demonstrated. Despite ready photoinactivation of chlorophenols in laboratory systems, conditions which prevent adequate ultraviolet penetration of water are frequently found in the environment; therefore, the major route of chlorophenol dissipation in water results from biodegradation.

Adsorption to suspended and/or bottom sediments may also play a role in the dissipation of chlorophenols from water. Laboratory studies indicate that in general monochlorophenols, dichlorophenols, and trichlorophenols are only weakly sorbed by soil particles. Thus, removal of these compounds from the aqueous system by sorption appears unlikely. On the other hand, pentachlorophenol may be strongly sorbed to certain soil types when conditions such as pH are appropriate. Dissipation of pentachlorophenol from aqueous systems through sorption is a likely possibility; however, the extent to which sorption contributes to the removal of pentachlorophenol from water remains speculative. The chemical similarity of the tetrachlorophenols to pentachlorophenol suggests that sorption may play a role in the dissipation of 2,3,4,6-tetrachlorophenol and other tetrachlorophenol isomers from aquatic systems.

Biodegradation is probably the major route of chlorophenol dissipation from aquatic environments. In view of the known microbial toxicity of chlorophenols, ready degradation of these compounds in aquatic environments probably requires acclimated or adapted bacterial strains. This expectation has been confirmed in experimental studies. Low concentrations of 2-chlorophenol added to domestic sewage were not removed during periods of 20 to 30 days; however, similar concentrations added to polluted river waters dissipated in 15 to 23 days. Other experiments have indicated that the removal of monochlorophenols from water requires the presence of specialized microflora. Activated sludge preparations acclimated to 2-chlorophenol rapidly degrade the compound. In a similar vein, 2,4-dichlorophenol is degraded rapidly in the presence of acclimated activated sludge.

The location of chlorine on the phenol nucleus affects degradation. The presence of a chlorine atom at the meta position of the phenol ring retards biodegradation. For example, 2,4,5-trichlorophenol is more persistent than 2,4,6-trichlorophenol. Limited data indicate that 2,4,5- and 2,4,6-trichlorophenol are degraded rapidly under specified conditions in activated sludge or aeration lagoon effluent. Although no data are available to indicate the persistence of 2,3,4,6-tetrachlorophenol in aquatic systems, soil suspensions have been tested for ability to degrade chlorophenols. These data may shed some light on the expected persistence of the various chlorophenol isomers in natural water-soil systems. Table A.7.1 indicates that 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol are persistent in the absence of acclimated microorganisms. When acclimated microorganisms are present, all chlorophenol isomers are degraded relatively rapidly, with pentachlorophenol being the most refractory.

Many investigations have shown that pentachlorophenol degradation does not occur readily in aquatic environments (Table A.7.1). However, other investigations have indicated that under appropriate conditions

TABLE A.7.1. DEGRADATION OF CHLOROPHENOLS IN ACCLIMATED ACTIVATED SLUDGE[a]

Compound	Ring degradation		Production of chloride ion	
	(%)	(days)	(%)	(days)
2-Chlorophenol	100	3	100	4
3-Chlorophenol	100	2	100	3
4-Chlorophenol	100	3	100	3
2,4-Dichlorophenol	100	5	100	5
2,5-Dichlorophenol	52	4	16	4
2,4,6-Trichlorophenol	100	3	75	3
Sodium pentachlorophenate	0	4	0	4
Dichloroquinone	100	1	50	3
2,5-Dichlorophenol benzoquinone	30	1	0	1

[a] Concentration of 100 mg/liter.

Source: Adapted from Ingols, Gaffney, and Stevenson, 1966, Table IV, p. 631. Reprinted by permission of the publisher.

pentachlorophenol is degraded rapidly in activated sludge or under laboratory conditions where suitably activated microbial populations are present. Under specified conditions, pentachlorophenol levels in water declined to negligible amounts in 48 hr, but other experiments indicated that substantial amounts of pentachlorophenol may remain in a laboratory system for more than 120 days. Thus, depending on environmental conditions, pentachlorophenol may degrade rapidly and pose little or no environmental hazard, or it may have marked longevity, thereby posing a potential hazard of indeterminate proportions. Despite the lack of data on the persistence of 2,4,5,6-tetrachlorophenol, this compound is speculated to behave in a manner similar to pentachlorophenol. None of the chlorophenols should be allowed to reach unacclimated bodies of water where their persistence may be considerably extended. The ecological effects resulting from their presence remain largely undefined.

A.7.3.3 Mobility and Persistence in Soil

The movement of chlorophenols in soil depends on the interaction and persistence of the chemicals. More persistent compounds have a greater chance to interact with the soil particles, particularly by the sorption process. The extent of sorption determines whether the chemicals are carried in association with eroded soils during overland flow or are leached through the soil profile during infiltration. Soil factors affecting sorption include pH, moisture, and clay and organic matter content. Sorption characteristics of chlorophenols other than pentachlorophenol have not been studied adequately. Based on limited data, 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5- and 2,4,6-trichlorophenol seem to be sorbed only weakly by soil particles. Thus, the potential for downward movement through the soil profile and for groundwater contamination exists. Severe cases of contamination due to infiltration of 2,4-D and 2,4-dichlorophenol into the groundwater have been reported. Two instances of groundwater pollution resulting from emptying wastes containing 2,4-D and 2,4-dichlorophenol into local sewage systems and from discharge of the compounds into lagoons have been reported. In view of the ready biodegradability of these compounds in soil and the very small quantities which are expected to contaminate soil in the routine usage of the herbicides 2,4-D and 2,4,5-T, groundwater contamination is not likely to occur. However, when highly concentrated solutions have been allowed to seep through the soil, contamination of groundwater has resulted. Pentachlorophenol, on the other hand, is tightly bound by soil particles and the likelihood of leaching through the soil profile into groundwater is reduced markedly. 2,3,4,6-Tetrachlorophenol may share this property with pentachlorophenol, but direct data are not available.

Biodegradation plays a crucial role in the dissipation of the chlorophenols from soil. Microbial decomposition in soil suspensions occurs rapidly for 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol, but 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol are more persistent (Table A.7.2). All chlorophenols persist longer in soil under conditions unfavorable for microbial growth and activity; the presence of acclimated populations of bacteria and fungi increases the degradation rate of these compounds. Under appropriate conditions, even the more persistent chlorophenols (2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol) are degraded. The rate of degradation depends on a wide variety of soil parameters. The complexity of the interactions between soil microbial populations and any given chlorophenol frequently precludes general predictions of persistence. For example, persistence of pentachlorophenol in soil ranges from 21 to 200 days, depending on the specific conditions at the site of application.

A.7.4 Waste Management

Chlorophenols are degraded readily in well-designed wastewater treatment plants. However, dumping or seepage of these compounds into unacclimated bodies of water or shock loading of sewage treatment plants may pose serious hazards. Wastewater treatment of chlorophenols involves relatively long retention times and precautions to avoid leaching of lower chlorophenol isomers through the soil profile into groundwater supplies. The distribution of chlorophenol-metabolizing microorganisms in the environment is not known, and further research in this area is warranted. The major ecological hazard is likely posed by highly chlorinated chlorophenols such as pentachlorophenol and tetrachlorophenols. In view of the fact that these are the most toxic chlorophenols, caution should be used in the disposal of these compounds to insure that large quantities do not reach sensitive areas of the environment.

TABLE A.7.2. MICROBIAL DECOMPOSITION OF CHLOROPHENOLS IN SOIL SUSPENSIONS

Compound	Time for complete dissipation (days)	
	Dunkirk silt loam[a]	Mardin silt loam[a]
Phenol	2	1
2-Chlorophenol	14	47
3-Chlorophenol	>72	>47
4-Chlorophenol	9	3
2-Bromophenol	14	*b*
3-Bromophenol	>72	*b*
4-Bromophenol	16	*b*
2,4-Dichlorophenol	9	5
2,5-Dichlorophenol	>72	*b*
2,4,5-Trichlorophenol	>72	>47
2,4,6-Trichlorophenol	5	13
2,3,4,6-Tetrachlorophenol	>72	*b*
Pentachlorophenol	>72	*b*
2,4-D	26	23
2,4,5-T	>205	>47
Silvex [2-(2,4,5-trichlorophenoxy) propionic acid]	>205	>47

[a] Substrate added, 50 mg/liter.
[b] No data.

Source: Adapted from Alexander and Aleem, 1961, Tables I and II, p. 45. Reprinted by permission of the publisher.

SECTION A.7

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A.8 ENVIRONMENTAL INTERACTIONS AND THEIR CONSEQUENCES

A.8.1 ENVIRONMENTAL CYCLING OF CHLOROPHENOLS

The sources, distribution, and fate of chlorophenols in soil, aquatic environments, and the atmosphere are discussed in Section A.7. A possible cycle of these chemicals in the environment showing likely sources of contamination, interaction, and sinks is depicted in Figure A.8.1. Caution should be used in interpretation of this cycle because the limited data available make it difficult to establish any pattern of distribution and flow of the chemicals into various parts of the environment.

Soil and aquatic systems could be contaminated through direct usage of chlorophenols or closely related compounds, discharge of industrial wastes and sewage, and chlorination of water containing phenols. Although no documentation is available on the relative contribution of each source, it is logical that contamination with 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol occurs mainly by application of the chemically related phenoxyalkanoic herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which may contain chlorophenols as contaminants and produce them as degradation products. 2,4,6-Trichlorophenol is used as a microbicide, and residues are expected to result from the usage and manufacture of the compound. Pentachlorophenol and 2,3,4,6-tetrachlorophenol have been used as broad-spectrum biocides and wood preservatives. 2,3,4,6-Tetrachlorophenol is used only sparingly, but pentachlorophenol is used extensively as a wood preservative and microbicide. Additionally, 2,3,4,6-tetrachlorophenol may be present as an impurity of technical-grade pentachlorophenol preparations. Leaching from preservative-treated items and dissemination of pentachlorophenol as a herbicide, molluscicide, algicide, or slimicide are likely sources for pentachlorophenol contamination of the environment. Chlorophenols are volatile; the degree of volatility decreases with increased chlorine substitution. Data on the presence of chlorophenols in the atmosphere and their subsequent redeposition during precipitation and fallout are extremely limited.

Chlorophenols undergo physicochemical and biological interactions with various components of the environment. The chemicals can be sorbed by soils, sediment, and airborne particulate material. Sorption plays a major role in the downward and overland movement of contaminants from soil surfaces. Tightly bound compounds tend to associate with the eroded soil particles during runoff, but weakly bound compounds may move with the water phase during runoff and infiltration. Limited evidence indicates that the sorption of 2-chlorophenol, 2,4-dichlorophenol, and perhaps the trichlorophenols is insignificant, suggesting that these compounds are mobile and would not be associated with particulate materials in the runoff process. On the other hand, 2,3,4,6-tetrachlorophenol and pentachlorophenol appear to be tightly bound to soil particles under most conditions. Thus, these compounds are expected to remain in the soil until biodegradation or other dissipation mechanisms remove them.

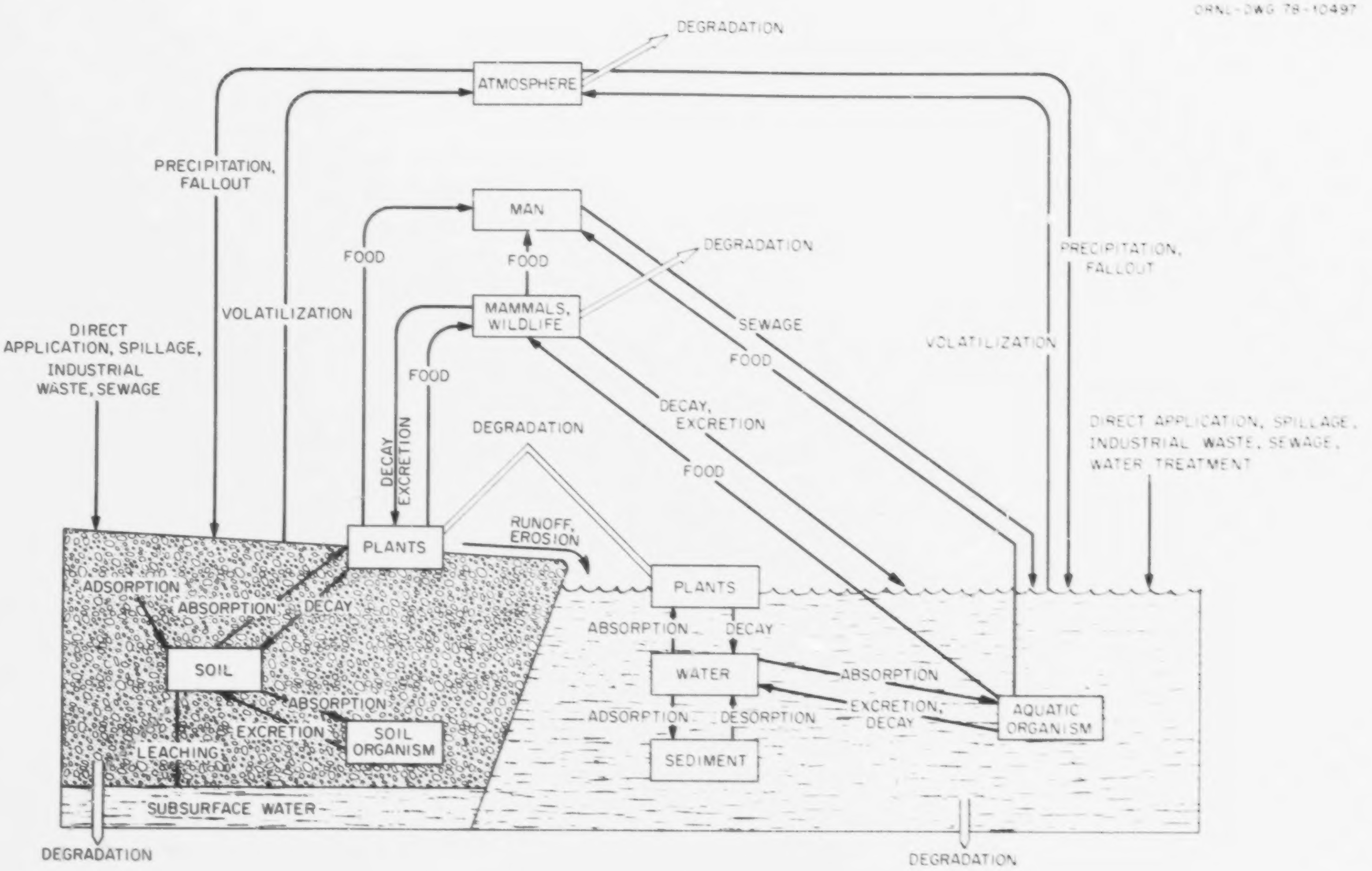
ORNL-DWG 78-10497
DEGRADATION
ATMOSPHERE
PRECIPITATION, FALLOUT
VOLATILIZATION
MAN
FOOD
FOOD
MAMMALS, WILDLIFE
DEGRADATION
FOOD
DECAY EXCRETION
SEWAGE
FOOD
DECAY, EXCRETION
FOOD
PRECIPITATION, FALLOUT
VOLATILIZATION
DIRECT APPLICATION, SPILLAGE, INDUSTRIAL WASTE, SEWAGE
DIRECT APPLICATION, SPILLAGE, INDUSTRIAL WASTE, SEWAGE, WATER TREATMENT
DEGRADATION
PLANTS
RUNOFF, EROSION
ADSORPTION
ABSORPTION
DECAY
SOIL
ABSORPTION
EXCRETION
SOIL ORGANISM
LEACHING
SUBSURFACE WATER
DEGRADATION
PLANTS
ABSORPTION
DECAY
WATER
ABSORPTION
EXCRETION, DECAY
AQUATIC ORGANISM
ADSORPTION
DESORPTION
SEDIMENT
DEGRADATION

Figure A.8.1 Possible cycling of chlorophenols in the environment.

The rates of chemical and biological transformation of chlorophenols determine their persistence and subsequent degree of movement in a particular segment of the environment. Chemical oxidation may occur during treatment of wastewater, and photodecomposition is possible when the compounds are present at water, soil, and airborne particulate surfaces exposed to sunlight. It has been shown, although data are limited and fragmentary, that each chlorophenol reviewed in this report can be assimilated by soil and aquatic microorganisms, absorbed by plants, and absorbed and/or ingested by mammals. Microbial degradation appears to be the principal mechanism of dissipation. The degree of metabolism and mode of distribution of absorbed or ingested chlorophenols in plants, wildlife, and mammals may influence possible bioaccumulation. Evidence suggests that pentachlorophenol may bioaccumulate in liver and kidney to a limited extent in terrestrial animals, and limited biomagnification almost certainly occurs in aquatic ecosystems. Information on the bioaccumulation of other chlorophenols is lacking. Bioaccumulated chlorophenols, if they persist, can be returned either to the soil and water system during decay and excretion or be consumed by higher organisms, including humans, through the food chain.

A.8.2 CHLOROPHENOLS IN FOOD

With the exception of pentachlorophenol, none of the chlorophenols examined in this report are used directly in agricultural production. In fact, the limited use of pentachlorophenol as a herbicide places it generally in the same category. Despite this lack of agricultural use, the compounds may find their way into crops (e.g., metabolism of chemically related herbicides by plants or animals). Degradation of lindane in rats results in the production of a large number of chlorophenol isomers (Figure A.8.2). The extent to which herbicides or other similar organic chemicals are degraded to form chlorophenols in the environment remains speculative. Present data indicate that these processes are likely to occur, but the extent to which these lower chlorophenols appear in foods is unknown. Large amounts of 2,4-D and 2,4,5-T must be consumed to cause detectable concentrations of chlorophenols in domestic animals; it seems highly unlikely that sufficient quantities of the phenoxyalkanoic herbicides would be ingested to cause a chlorophenol problem.

Transfer of the chlorophenols to humans through the food chain is remote, with the exception of pentachlorophenol. Small amounts of pentachlorophenol have been detected in food samples analyzed by the Food and Drug Administration of the U.S. Department of Health, Education, and Welfare. Thus, some portion of the nation's population is exposed to low levels of pentachlorophenol through food consumption, but it is impossible to describe the extent of the associated hazards. The source of the pentachlorophenol in food is unclear. Contact of produce and meat with pentachlorophenol-treated items during packaging and distribution may in part be responsible for low pentachlorophenol levels in food products. Also, the widespread use of pentachlorophenol as a wood preservative may allow some degree of absorption by plants and domestic animals in contact with treated lumber. Whatever the source, no chronic health problems have been associated with the low levels of pentachlorophenol detected in human urine.

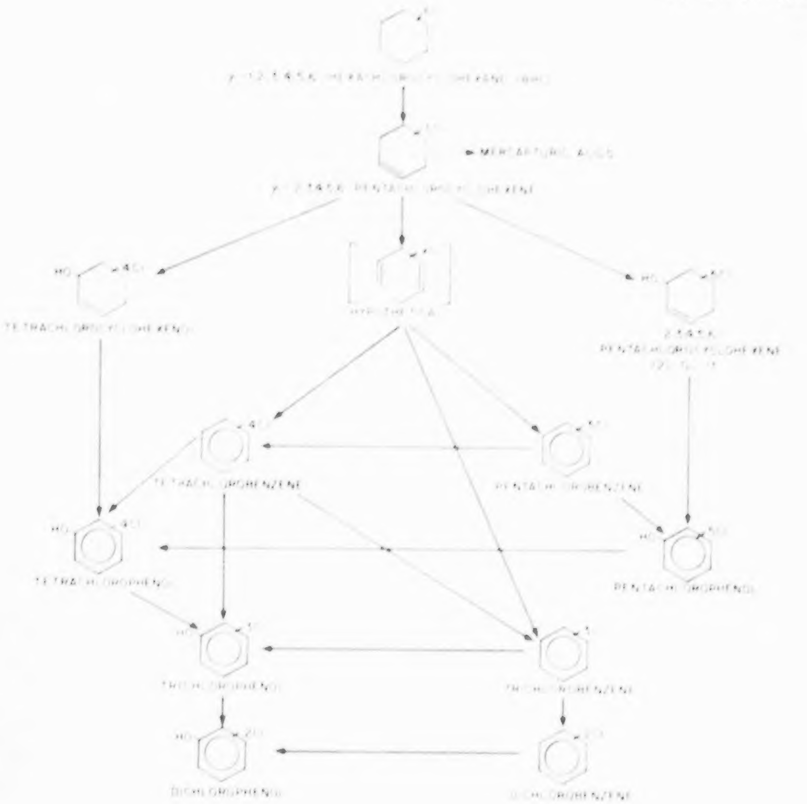
MERCAPTURIC ACIDS
HO
TETRACHLOROCYCLOHEXENOL
HYPOTHETICAL
2,3,4,5,6-
PENTACHLOROCYCLOHEXENE
TETRACHLOROBENZENE
PENTACHLOROBENZENE
TETRACHLOROPHENOL
PENTACHLOROPHENOL
TRICHLOROPHENOL
TRICHLOROBENZENE
DICHLOROPHENOL
DICHLOROBENZENE

Figure A.8.2. Proposed metabolic pathway for lindane in rats. Source: Adapted from Engst et al., 1976, Figure 12, p. 113. Reprinted by courtesy of Marcel Dekker, Inc.

Pentachlorophenol appears to accumulate in the tissues of aquatic organisms exposed to ambient levels of the substance. The extent to which pentachlorophenol bioconcentrates or biomagnifies in aquatic food chains is unknown and this question warrants further research.

In summary, the available data on environmental contamination by chlorophenols preclude the formulation of definitive conclusions regarding the effects of these compounds on humans. The extent to which the wide distribution of chlorophenols, particularly pentachlorophenol, affects human health and welfare and the integrity of the environment requires further investigation so that policymakers as well as the public can be fully informed concerning the environmental impact of chlorophenol use and can determine if additional regulatory measures are warranted.

SECTION A.8

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PART B

2-CHLOROPHENOL AND 2,4-DICHLOROPHENOL

B.1 SUMMARY

B.1.1 DISCUSSION OF FINDINGS

2-Chlorophenol, a light amber-colored liquid at room temperature, has a pungent medicinal odor. It is slightly soluble in water but it is readily soluble in organic solvents and aqueous alkaline solutions. The compound possesses a substantial vapor pressure and readily volatilizes with steam, even from aqueous alkaline solutions. This characteristic may contribute to its distribution in the environment. Most 2-chlorophenol produced is used as an intermediate in the synthesis of higher chlorophenols. It has been used as a polymer intermediate in the manufacture of fire-retardant varnishes and as an aminizing agent for cotton fabric to provide rot resistance and crease recovery.

2-Chlorophenol may be converted to higher chlorophenols (particularly 2,4- and 2,6-dichlorophenol) during chlorination of drinking water or wastewater. The resulting chlorophenols contribute significantly to the objectionable phenolic odor and taste of water, making it unfit for human consumption. Another important reaction of 2-chlorophenol is increasing ionization of the compound at pH values above its pK value. The resulting ions may form salts with alkali metals and these compounds, being more soluble in water, may be transported more easily in the ecosystem.

2,4-Dichlorophenol exists as colorless crystals at room temperature. Like 2-chlorophenol, its solubility in water is very low, but its solubility in organic solvents and in aqueous alkaline solutions is high. 2,4-Dichlorophenol is much less volatile than 2-chlorophenol, and transport through the atmosphere is less likely to occur. 2,4-Dichlorophenol serves primarily as a starting material in the manufacture of other compounds, particularly pesticides. The most important use of 2,4-dichlorophenol is in the production of 2,4-dichlorophenoxyacetic acid (2,4-D). Other commonly used pesticides such as sesone and nitrofen are also manufactured from 2,4-dichlorophenol. Widespread use of these pesticides may contribute to 2,4-dichlorophenol contamination of the environment as a result of pesticide degradation. Some metal salts of 2,4-dichlorophenol have been used as germicides and antiseptics.

2,4-Dichlorophenol undergoes reactions similar to those of 2-chlorophenol in the environment. Dichlorophenols have been implicated as compounds partially responsible for the objectionable phenolic odor and taste in water. In addition, 2,4-dichlorophenol may be chlorinated during chlorination of drinking water or wastewater, resulting in the formation of higher chlorophenols which are more potent in causing objectionable taste and odor. Solubility and sorption properties of 2,4-dichlorophenol may be altered by pH-ionization effects.

A number of analytical techniques have been used in the identification and quantification of 2-chlorophenol and 2,4-dichlorophenol in samples of soil, water, and biological tissues. Gas-liquid chromatography (employing either electron-capture or flame-ionization detectors) appears to be the best method for separation, identification, and quantification of low levels of chlorophenols found in a wide variety of environmental samples. Colorimetric methods, although simple and rapid, lack specificity and thus are only useful for gross determination of chlorophenols. Other methods of detection, including thin-layer chromatography, infrared and ultraviolet spectrophotometry, and mass spectrometry, provide specific identification but are useful only for determination of relatively large concentrations.

Both 2-chlorophenol and 2,4-dichlorophenol are bactericidal, fungicidal, and algicidal. 2,4-Dichlorophenol has been used in the past as a bactericide, but its use has now been supplanted by more effective agents. 2,4-Dichlorophenol is generally a more effective microbicide than 2-chlorophenol. It has been proposed that the bactericidal action of these compounds stems from effects on the lipids of bacteria, causing cell wall damage, changes in permeability, and perhaps enzyme inhibition. In general, bactericidal and algicidal potency increases with increased chlorine substitution of the parent phenol up to the trichlorophenol isomers.

2-Chlorophenol and 2,4-dichlorophenol are not highly toxic to plants. 2,4-Dichlorophenol may appear in vascular plants following application of 2,4-D. Large amounts of 2,4-dichlorophenol are sorbed by the roots of oats and soybeans grown in 2,4-dichlorophenol-treated soils or nutrient solutions. Although small quantities of the compound are detected in the shoots of treated plants, transport does not appear to occur readily. 2,4-Dichlorophenol does not concentrate in the seeds of soybeans or oats. 2,4-Dichlorophenol applied to leaves does not appear to be translocated.

Biotransformation of lower chlorophenols by vascular plants is not well studied. It has been proposed that conjugation specifically by glycoside formation and immobilization of the compound may occur when 2-chlorophenol or 2,4-dichlorophenol are absorbed by plants. A glycoside of 2-chlorophenol, β-*O*-chlorophenyl gentiobioside, has been isolated from the roots of tomato plants grown in culture media containing 2-chlorophenol. The mechanism of toxic action of lower chlorophenols to vascular plants is not well studied. 2-Chlorophenol and 2,4-dichlorophenol are capable of inhibiting certain plant enzyme systems. No information exists on the phytotoxic effects of 2-chlorophenol in vascular plants, but 2,4-dichlorophenol has been shown to inhibit root growth in flax seedlings, cell expansion in wheat coleoptiles, and the growth-stimulating effect of indoleacetic acid in wheat. 2,4-Dichlorophenol clearly induces increased frequency of mitotic abnormalities in the European broad bean. Effects on the productivity of treated plants are minimal, however, and the significance of the mitotic effect to domestic and wild plants is not known.

Biological impacts of 2-chlorophenol and 2,4-dichlorophenol in birds and mammals are generally unknown. Sheep and cattle ingesting 2,4-D in large amounts generally exhibited a weakened condition. Small doses were well tolerated with no detectable adverse effect. These animals metabolized 2,4-D to form 2,4-dichlorophenol, which was detected in the liver and kidney. 2,4-Dichlorophenol was retained in the liver over a one-week withdrawal. The significance of 2,4-dichlorophenol in producing clinical toxicity in this study is unknown. 2,4-Dichlorophenol has also been detected in laying hens administered nemacide. Relatively high levels of the compound were detected in the liver and the egg yolk, but no residues were found in the fat or in breast muscle. Substantial levels of 2,4-dichlorophenol were detected in the liver for as long as 21 days following removal from treated feed. No signs of toxicity were noted in the hens.

2-Chlorophenol is toxic to aquatic organisms. Median lethal tolerance limits for fish range from 2.6 to 8.2 mg/liter. 2,4-Dichlorophenol affects oxygen consumption and cell division in fertilized sea urchin eggs at a concentration of 10 mg/liter. The 48-hr median lethal tolerance limit of 2,4-dichlorophenol to daphnia is reportedly 2.6 mg/liter. The mechanism of action of these compounds in aquatic organisms is speculative. Both compounds can inhibit oxidative phosphorylation in higher animals, and it has been proposed that this effect also contributes to toxicity in aquatic organisms.

2-Chlorophenol and 2,4-dichlorophenol are corrosive to the skin of humans and experimental animals. Both compounds may be absorbed directly through the skin, but 2-chlorophenol is more easily absorbed by this route. 2-Chlorophenol administered subcutaneously, intravenously, intraperitoneally, or orally is toxic to experimental animals. Median lethal doses for 2-chlorophenol range from 100 to 800 mg/kg body weight. Oral, intraperitoneal, or subcutaneous administration of 2,4-dichlorophenol to experimental animals results in LD_{50} values of 430 to 1730 mg/kg. Chronic feedings of 2,4-dichlorophenol to mice at levels as high as 100 mg/kg daily had no discernable effect over a six-month period. 2-Chlorophenol has not been chronically administered to experimental animals. No documented cases of human poisoning by either compound were found.

2-Chlorophenol and 2,4-dichlorophenol are apparently excreted primarily in the urine, and the formation of conjugates (particularly with sulfuric and glucuronic acids) has been demonstrated. 2,4-Dichlorophenol and its conjugates have been detected in the urine of experimental animals administered γ-1,2,3,4,5,6-hexachlorocyclohexane (lindane). Elimination of 2,4-dichlorophenol following its production from other organic substances appears to be relatively rapid.

As mentioned above, 2,4-dichlorophenol appears in mammals as a result of metabolism of other organic compounds (e.g., lindane and nemacide). Similarly, 2-chlorophenol has been detected as a metabolite of organic compounds, including chlorobenzene isomers. Exposure of animals or humans to these precursor compounds is more probable than direct exposure to either 2-chlorophenol or 2,4-dichlorophenol. Neither compound is used widely, and exposure is therefore likely to be limited to workers in the manufacturing industry. Exposure of the general population is more likely to occur through contact with compounds such as lindane or nemacide, which have been widely used as pesticides.

No physiological or biochemical role for 2-chlorophenol or 2,4-dichlorophenol in any biological system has been found; therefore, it is unlikely that a nutritional requirement for either of these compounds exists. The mechanism of toxic action of 2-chlorophenol and 2,4-dichlorophenol on higher organisms is speculative. Both compounds have inhibited oxidative phosphorylation in in vitro tests. They are relatively weak uncouplers of oxidative phosphorylation, but if dosages are high enough, this effect can cause death. 2,4-Dichlorophenol also affects various enzyme systems in the cell.

Experimental animals fatally poisoned by 2-chlorophenol usually exhibit tremors and convulsions, but symptoms of 2,4-dichlorophenol poisoning are vague and do not include nervous effects. The biological basis for these different clinical pictures is not known.

2-Chlorophenol and 2,4-dichlorophenol promote the appearance of benign and malignant skin tumors in mice following a single initiating dose of the compound dimethylbenzanthracene. No evidence exists, however, that either compound causes tumors in the absence of an initiator. Despite this fact and the questionable hazard to humans, protracted skin contact is not recommended because of the potential for tumor formation and because both compounds are primary skin irritants.

Only one study has suggested that 2,4-dichlorophenol may have adverse effects in humans. Acquired chloracne and porphyria (indicative of some liver damage) have been reported among workers involved in the manufacture of 2,4-dichlorophenol and 2,4,5-trichlorophenol. The wide variety of chemicals to which these workers were exposed precludes an absolute identification of the responsible substance, but available evidence indicates that tetrachlorodibenzo-*p*-dioxin was involved.

Monitoring data on the presence of 2-chlorophenol or 2,4-dichlorophenol in air, water, or soil are not available. The moderate volatility of these compounds suggests that volatilization is a mechanism for dispersing the chemicals into the atmosphere. Incineration might also generate volatile products to the atmosphere through the burning of containers and trash containing chlorophenols. Movement, persistence, and fate of the compounds in the atmosphere are not understood. Possibly, photodecomposition contributes to their dissipation.

Contamination of water with 2-chlorophenol and 2,4-dichlorophenol could arise from (1) chlorination of phenols present in natural water and in primary and secondary effluents of waste treatment plants, (2) direct addition of the chemicals from industrial sources or as contaminants or degradation products of 2,4-D used for aquatic weed control, and (3) wet and dry atmospheric fallout. Additionally, runoff from agricultural land where 2,4-D or similar herbicides are used could be an important source of chlorophenols in aquatic environments. 2-Chlorophenol and 2,4-dichlorophenol may be transported in aquatic environments in the dissolved form, associated with suspended matter and bottom sediments, or absorbed in biological tissues. Both 2-chlorophenol and 2,4-dichlorophenol appear to dissipate rather rapidly in aquatic systems. Microorganisms apparently play a crucial role in the degradation of these compounds, although it is known that 2,4-dichlorophenol undergoes photolysis when exposed to ultraviolet light. Thus, solar radiation may contribute to the degradation of the compound in water of sufficient clarity to allow penetration of solar radiation.

Neither 2-chlorophenol nor 2,4-dichlorophenol appear to pose a serious hazard to waste treatment facilities which are normally exposed to these compounds; they are readily degraded by activated sludge and by microbial populations in aeration lagoon effluent. Some investigations have shown that a specialized microflora is necessary for rapid dissipation of the compounds. Additionally, limnological factors such as oxygen depletion affect degradation rates. Some hazard therefore exists if wastes containing high levels of these compounds are discharged to an unacclimated body of water or sewage treatment facility. 2-Chlorophenol and 2,4-dichlorophenol are apparently totally degraded under optimum conditions within five days; pH, temperature, and concentration of the compounds affect the speed of degradation.

The primary source of soil contamination by chlorophenols is probably through the application of 2,4-D and its derivatives on croplands, although atmospheric fallout or washout may also contribute to contamination. The herbicide 2,4-D and its derivatives are used widely. In 1971, U.S. farmers applied almost 16 million kilograms of 2,4-D, which represents 15% of all organic herbicide usage. 2,4-Dichlorophenol may be applied directly to croplands as an impurity in 2,4-D preparations. Amounts ranging from 70 to 4500 mg/kg of 2,4-D have been reported. Therefore, 2,4-dichlorophenol (and possibly 2-chlorophenol) generated from herbicide applications has the potential to accumulate depending on environmental conditions and the presence or absence of microorganisms capable of its further degradation. Groundwater contamination with 2,4-D and 2,4-dichlorophenol has been reported in two serious cases. Wastewaters containing 2,4-D and 2,4-dichlorophenol were dumped into local sewage systems with the resultant phenolic tastes and odors downstream in shallow wells; the problem persisted for three years. In Colorado, 2,4-D was manufactured and wastes were discharged into lagoons from 1943 to 1957. Groundwater contamination was first reported in 1951 when crops were damaged by irrigation well water. Seven to eight years were required for the contaminant to migrate 5.6 km and to affect an area of 16.8 km^2.

Present data indicate that 2-chlorophenol and 2,4-dichlorophenol are not bound tightly to soil and that sorption, when it occurs, is likely to be weak. Desorption and downward movement through the soil profile may occur; however, contamination of groundwater is expected to occur only when highly concentrated solutions are applied to soil. Evidence suggests that microorganisms found commonly in soil are capable of degrading both 2-chlorophenol and 2,4-dichlorophenol. Microbial decomposition appears to be the dominant dissipation mechanism for these compounds in soil. Percolation experiments have indicated that 2-chlorophenol may be degraded within ten days, and 2,4-dichlorophenol is similarly short-lived in soils. Both bacterial and fungal species are capable of degrading chlorophenols, and a number of bacterial species may utilize the lower chlorophenols as their sole source of organic carbon and energy. Whether or not fungal strains are capable of utilizing lower chlorophenols as a sole carbon source is not known.

Food contamination with 2-chlorophenol and 2,4-dichlorophenol appears to be minimal; thus, transfer to humans through the food chain is apparently remote. The direct exposure of humans to these chemicals during manufacture constitutes the major hazard. Widespread distribution and accumulation of 2-chlorophenol and 2,4-dichlorophenol in the environment is unlikely because these compounds tend to degrade readily in soil and aquatic systems. No data are available, however, to conclusively prove these contentions. More definitive investigations are needed to assess the environmental hazards associated with the usage of the compounds. Accumulation in fish and other aquatic organisms exposed to waters contaminated with the chemicals has not been investigated. The widespread use of chlorination for waste treatment and water purification also must be questioned. The presence of lower chlorophenols may allow the formation of the more dangerous highly chlorinated phenols in wastewater or drinking water.

B.1.2 CONCLUSIONS

1. 2-Chlorophenol can be converted to higher chlorophenols (including 2,4-dichlorophenol) during chlorination of drinking water or wastewater, imparting objectionable phenolic odor and taste to the water.

2. 2,4-Dichlorophenol may be converted to higher chlorophenols during chlorination of drinking water or wastewater.

3. Gas-liquid chromatography is the best method for separation, identification, and quantification of low levels of 2-chlorophenol and 2,4-dichlorophenol in environmental samples.

4. 2-Chlorophenol and 2,4-dichlorophenol are bactericidal, fungicidal, and algicidal.

5. 2-Chlorophenol and 2,4-dichlorophenol are not highly toxic to plants, terrestrial animals, or humans.

6. 2,4-Dichlorophenol may appear in domestic animals following absorption of the chemically related phenoxyalkanoic herbicides.

7. 2-Chlorophenol and 2,4-dichlorophenol are moderately toxic to aquatic organisms; LC_{50} values range from 2.6 to 8.2 mg/liter.

8. Elimination of 2-chlorophenol or 2,4-dichlorophenol occurs primarily through the urine, and the presence of sulfuric and glucuronic acid conjugates has been reported.

9. Chronic toxicity of 2-chlorophenol and 2,4-dichlorophenol has not been documented.

B.2 CHEMICAL AND PHYSICAL PROPERTIES AND ANALYSIS

B.2.1 PHYSICAL PROPERTIES

2-Chlorophenol is a light amber-colored liquid at room temperature and has a pungent, medicinal odor. It is slightly soluble in water but is readily soluble in several organic solvents and aqueous caustic alkaline solutions. It volatilizes with steam, even from aqueous alkaline solutions. Table A.2.1 lists the important physical properties of 2-chlorophenol.

2,4-Dichlorophenol exists as colorless crystals at room temperature. It has an unpleasant and persistent odor similar to iodoform. Its solubility in water is very low, although it is easily soluble in many organic solvents and is also soluble in aqueous alkaline solutions. This compound generally behaves as a weak acid and volatilizes with steam, but unlike 2-chlorophenol, it is not volatile from aqueous alkaline solutions. Some of the important physical properties of 2,4-dichlorophenol are given in Table A.2.1.

2,4-Dichlorophenol has higher boiling and melting points than 2-chlorophenol. This wide variation in boiling points is useful in separating the two compounds by fractional distillation. Another factor that can be utilized in separating the monochlorophenol from the dichlorophenol is volatilization. Both chemicals are volatile, but 2-chlorophenol has a much higher vapor pressure (1 mm Hg at 12.1°C) than 2,4-dichlorophenol (1 mm Hg at 53°C), making it more susceptible to volatilization.

B.2.2 MANUFACTURE

2-Chlorophenol can be synthesized by diazotization of 2-chloroaniline, direct chlorination of phenol, or hydrolysis of 1,2-dichlorobenzene (Doedens, 1963). The most commonly used process in the commercial production of 2-chlorophenol is direct chlorination of phenol. Direct chlorination is carried out by passing gaseous chlorine into molten phenol at temperatures of 50°C to 150°C. This process leads to the formation of both 2- and 4-chlorophenol and the isomers are separated by fractional distillation because the difference in boiling points is greater than 40°C. Most of the 2-chlorophenol used commercially in the United States is recovered as a by-product from the manufacture of 4-chlorophenol by direct chlorination of phenol.

The production of 2,4-dichlorophenol by direct chlorination of phenol with 2 moles of chlorine was patented by Imperial Chemical Industries in 1948 (Doedens, 1963). This method involves passing chlorine into molten phenol at 80°C to 100°C until a product with a melting point of 34°C to 36°C is obtained. The reaction results in the formation of small amounts of 2,6-dichlorophenol and 2,4,6-trichlorophenol. 2,4-Dichlorophenol can be isolated in a yield of 80% to 90%. Another method of preparing 2,4-dichlorophenol is the further chlorination of 2- or 4-chlorophenol. Chlorination of 2-chlorophenol yields a mixture of 2,4- and 2,6-dichlorophenol.

10. 2-Chlorophenol and 2,4-dichlorophenol promote the appearance of tumors in mice following an initiating dose of dimethylbenzanthracene, but neither compound causes tumors in the absence of an initiator.

11. Relatively rapid degradation of 2-chlorophenol and 2,4-dichlorophenol occurs in aquatic systems, including activated sludge and aeration lagoons, but a specialized microflora is necessary for optimum degradation rates of the compounds.

12. The primary source for soil (and perhaps water) contamination by lower chlorophenols is likely degradation of chemically related phenoxyalkanoic herbicides such as 2,4-D.

13. 2-Chlorophenol and 2,4-dichlorophenol are not tightly bound to soil particles, and groundwater contamination may result when large amounts (or highly concentrated solutions) of the compounds are dumped into local sewage systems or retained in waste lagoons.

14. Microbial decomposition readily dissipates 2-chlorophenol and 2,4-dichlorophenol from soils.

15. Food contamination with 2-chlorophenol and 2,4-dichlorophenol is minimal and transfer to humans through the food chain appears to be remote.

16. The possible accumulation of 2-chlorophenol and 2,4-dichlorophenol by fish and other aquatic organisms has not been investigated.

High-purity 2,4-dichlorophenol may be manufactured through chlorination of 4-chlorophenol by controlling the chlorine ratio to avoid formation of trichlorophenols.

B.2.3 USES

Most of the 2-chlorophenol produced in the United States is used as an intermediate in the synthesis of higher chlorophenols (Doedens, 1963). It can be used as a polymer intermediate in the manufacture of fire-retardant varnishes and as an aminizing agent for cotton fabric to provide rot resistance and crease recovery. Its use in fire-retardant products may now be insignificant because bromide compounds have largely been utilized as replacements. Although 2-chlorophenol possesses pesticidal and bactericidal properties, its use has been supplanted by more effective higher chlorophenols (Dow Chemical Company, 1977).

2,4-Dichlorophenol is primarily used as a starting material in the manufacture of pesticides (Doedens, 1963; Dow Chemical Company, undated). Its most important use is in the production of 2,4-dichlorophenoxyacetic acid (2,4-D). This compound and its derivatives have been used widely as hormone-type herbicides. Other herbicides produced with 2,4-dichlorophenol as an intermediate are sesone (sodium 2,4-dichlorophenoxy-ethyl sulfate) and nitrofen (2,4-dichlorophenyl 2-nitrophenyl ether). The dialkyl phosphorothioates and aryl and alkyl sulfonates of 2,4-dichlorophenol have been used as insecticides, nematocides, and miticides. For example, nemacide [*O*-(2,4-dichlorophenyl)*O*,*O*-diethyl phosphorothioate] is used both as an insecticide and nematocide, and Genite-EM-923 (2,4-dichlorophenyl benzenesulfonate) is used as a miticide. The metal salts of 2,4-dichlorophenol are used as germicides and antiseptics. 2,4-Dichlorophenol also has been used as a raw material to form polyether carbonate and polyester films (Dow Chemical Company, undated).

B.2.4 CHEMICAL REACTIVITY

2-Chlorophenol can undergo several chemical reactions (Doedens, 1963). Because the phenolic group activates the aromatic ring toward electrophilic substitution, many ring-substitution reactions are possible. Among the more important ones are further chlorination to 2,4- and 2,6-dichlorophenol, bromination, iodation, and nitration to monosubstituted and disubstituted derivatives. Condensation reactions of 2-chlorophenol with formaldehyde produce phenolic resin intermediates or methylene bridged polymers. The phenolic group behaves like phenol in etherification reactions, Claisen condensations, allylic rearrangements, and esterification with phosphorous oxychlorides. Under proper conditions, the hydroxyl group may ionize, and in the presence of alkali metals, metallic salts are formed.

Substitution occurs in the aromatic ring of 2,4-dichlorophenol (Doedens, 1963). It undergoes halogenation and nitration readily with substitution in the 6 position. Further chlorination of 2,4-dichlorophenol yields 2,4,6-trichlorophenol. Reaction with formaldehyde yields condensation products that are methylene bridged dimers. The phenolic group of 2,4-dichlorophenol reacts with acyl chlorides, ketenes, alkyl halides, sulfonyl halides, and oxides. Salt formation is accomplished easily by reaction with alkali metals.

Perhaps the most important reaction of the phenolic group of 2,4-dichlorophenol is in the synthesis of 2,4-D. Commercial production of 2,4-D involves reacting 2,4-dichlorophenol with monochloroacetic acid in the presence of sodium hydroxide. Other pesticides are also produced by reaction of the phenolic group. Reactions of chloronitrobenzene, ethyl sodium sulfate, and diethyl chlorothiophosphate with 2,4-dichlorophenol produce nitrofen, sesone, and nemacide respectively.

Most of the chemical reactions described can only be performed under controlled laboratory conditions. Two reactions may be particularly important in the environment – the conversions of 2-chlorophenol to 2,4- and 2,6-dichlorophenol and of 2,4-dichlorophenol to 2,4,6-trichlorophenol during chlorination of drinking water or wastewater. Dichlorophenols have been implicated as the compounds contributing largely to the objectionable phenolic odor and taste of water (Burttschell et al., 1959; Baker, 1965). Burttschell et al. (1959) reported that the threshold odor and taste concentrations for 2,4-dichlorophenol range from 2 to 8 µg/liter. Because 2-chlorophenol and 2,4-dichlorophenol have some weak acid characteristics, they can be ionized slowly in aqueous solutions. At pH values near or above their pK_a values, they may exist in the ionized form and may form salts in the presence of high concentrations of alkali metals. The salt forms of these chlorophenols are more soluble in water, which may favor their transport in solution in the ecosystem. The molecular form (non-ionized) of the two compounds may prevail at low pH values. The reactive form of the compounds (i.e., ionized or molecular) could play a role in the sorption of chlorophenols in soil, sediments, or particulate matter.

B.2.5 TRANSPORT AND TRANSFORMATION IN THE ENVIRONMENT

Transport mechanisms and transformation of 2-chlorophenol and 2,4-dichlorophenol in air, soil, and aquatic environments are discussed in detail in Section B.7. The summary presented below gives a perspective on the possible relationships of the physical and chemical properties of the chemicals to their interactions with different segments of the environment.

B.2.5.1 Air

Both 2-chlorophenol and 2,4-dichlorophenol are highly volatile, and volatilization may be the major dispersal mechanism of the chemicals in the atmosphere. However, because monitoring data indicating their presence are not available, transportation and transformation mechanisms in the atmosphere cannot be determined.

B.2.5.2 Aquatic Environment

2-Chlorophenol and 2,4-dichlorophenol may exist in the aquatic environment in the dissolved form, may be associated with suspended matter and bottom sediments, or may be absorbed in biological tissues. Metal salts of these compounds have greater water solubility, and if they are introduced or formed in situ, they would exist primarily in the dissolved form. Because chlorophenols are weak acids, they tend to ionize, depending on the pH of the system. They are nonionized in aqueous solution with pH lower than 5 and become increasingly dissociated as the pH rises (Cserjesi, 1972). The degree of dissociation could determine their sorption. Limited information indicates that they may not be sorbed significantly by colloids present in the aquatic environment. Hydrological factors such as current pattern and mixing and degradation and migration of organisms affect the movement of these chemicals.

A few investigations indicate that the dissipation of 2-chlorophenol (Ettinger and Ruchhoft, 1950) and 2,4-dichlorophenol (Aly and Faust, 1964) is largely microbiological. Persistence appears to be short, but limnological factors such as oxygen deficiency may delay degradation (Aly and Faust, 1964). Microorganisms found in activated sludge and waste lagoons degrade the two chemicals rather readily (Sidwell, 1971; Nachtigall and Butler, 1974).

2,4-Dichlorophenol undergoes photolysis when it is exposed to ultraviolet light (Aly and Faust, 1964). This process may be an important natural mechanism for its removal from water. Whether the amount of ultraviolet light from solar radiation is sufficient to cause photolytic reaction under natural conditions has not been determined.

B.2.5.3 Soils

The mobility of 2-chlorophenol and 2,4-dichlorophenol in soils may be influenced largely by sorption. The extent of sorption determines whether the chemicals are carried with soil during erosion or move through the soil profile during infiltration. Aly and Faust (1964) reported that only a small amount of 2,4-dichlorophenol was sorbed by clay minerals. Soil organic matter plays a major role in the sorption of many organic pesticides (Weber, 1972). It is generally accepted that although acidic pesticides such as 2,4-D are sorbed by organic matter, the sorption bond strength is weak. 2,4-Dichlorophenol is probably sorbed in a similar manner as 2,4-D, which makes it potentially susceptible to downward movement in soil with water.

There is strong evidence for the participation of microorganisms in the dissipation of 2-chlorophenol and 2,4-dichlorophenol in soils. Bacteria isolated from soils, including those known to degrade phenoxyacetic herbicides, are capable of metabolizing the compounds (Bollag, Helling, and Alexander, 1968; Evans et al., 1971; Spokes and Walker, 1974). Limited information indicates short persistence of the chemicals in soil (Walker, 1954; Alexander and Aleem, 1961).

B.2.6 ANALYSIS

Because the sample preparation, isolation, and determination of 2-chlorophenol and 2,4-dichlorophenol are the same, their analyses will be discussed concurrently.

B.2.6.1 Sampling, Storage, and Preservation

One of the most important considerations in the analysis of chlorophenols is sample collection, handling, and storage. The importance of proper sampling and preservation is underscored by the fact that present methods allow analyses at the nanogram level. Efforts should be made to eliminate contamination of samples and degradation of compounds. Information dealing specifically with chlorophenols does not exist; however, sampling and storage information for pesticides in *Standard Methods for the Examination of Water and Wastewater* (Rand, Greenburg, and Taras, 1976) and in a report by Chesters, Pionke, and Daniel (1974) may provide useful guidelines.

In sampling air, water, soil, and biological tissues, representative samples must be obtained. For any medium sampled, the reaction of the compounds in relation to the characteristics of the medium should be reviewed, and the physical and chemical characteristics of the sampling area should be investigated. The number of samples collected depends on the area and size of the medium to be sampled and the objectives of the study. Glass or plastic containers can be used to hold samples, but samples analyzed for organic compounds are best held in glass containers to prevent adsorption on the walls of the container.

Ideally, samples should be extracted and analyzed immediately after collection. If analysis is delayed, samples must be preserved to prevent biochemical and chemical changes. The commonly used method for samples not analyzed upon receipt is storage at 2°C to 4°C or at freezing temperatures. Chemical preservatives should be used only when they will not interfere with the ultimate analysis. Acidification with H_3PO_4 and $CuSO_4$ treatment followed by refrigeration at 5°C to 10°C has been recommended for the preservation of wastewater samples for phenol analysis (Rand, Greenberg, and Taras, 1976).

B.2.6.2 Sample Preparation for Analysis

Regardless of the kind of sample analyzed for 2-chlorophenol and 2,4-dichlorophenol, some form of sample preparation is necessary to obtain a reliable and accurate determination. This preparation involves isolation and cleanup methods (Table B.2.1). Isolation methods include blending, liquid-liquid extraction, and distillation. Direct-aqueous-injection gas-liquid chromatography techniques have been reported (Baker, 1966; Baird et al., 1974), but this method is limited to "clean" water samples. Complex samples such as soils and biological tissues need vigorous isolation and cleanup processes prior to chlorophenol determination.

The extraction technique used depends on the kind of samples analyzed. For biological tissues (i.e., plant and animal tissues), blending with solvent or hydrolysis with acid and alkali solutions prior to solvent extraction has been successful (Sherman, Beck, and Herrick, 1972; Steen et al., 1974; Clark et al., 1975). Chlorophenols in aqueous samples such as urine, milk, and bacterial cultures can be extracted directly after acidification (Loos, Roberts, and Alexander, 1967; Bjerke et al., 1972; Kuhihara and Nakajima, 1974; Nakagawa and Crosby, 1974; Rand, Greenberg, and Taras, 1976). In some instances, steam distillation with or without further extraction is sufficient (Loos, Roberts, and Alexander, 1967; Zigler and Phillips, 1967; Evans et al., 1971; Rand, Greenberg, and Taras, 1976). Fritz and Willis (1973) reported the separation of phenolic compounds, including 2-chlorophenol and 2,4-dichlorophenol, using high-pressure liquid chromatography with a polyacrylate resin column. The isolation and concentration of phenolic contaminants in water has also been accomplished by freeze-drying (Baker, 1966) and by adsorption on activated carbon followed by chloroform extraction (Eichelberger, Dressman, and Longbottom, 1970). Among the organic solvents used for the extraction of 2-chlorophenol and 2,4-dichlorophenol are hexane, ethyl acetate, ethyl ether, diethyl ether, carbon disulfide, methylene chloride, benzene, and petroleum ether.

The ultimate objective of sample preparation is to eliminate interferences so that samples are adequately clean for the determination and identification of the compound of interest. This procedure is particularly important with very low levels of 2-chlorophenol and 2,4-dichlorophenol in the environment. Liquid-liquid extraction and distillation could provide a means of eliminating interferences and concentrating samples. However, these processes are nonspecific, and other organic compounds may be coextracted or codistilled with the chlorophenols, causing interference problems; therefore, further cleanup must be performed on the extract or distillate. Cleanup methods are indicated in Table B.2.1. The most commonly used method is column chromatography (Bjerke et al., 1972; Shafik, Sullivan, and Enos, 1973; Steen et al., 1974). In this technique, extracts are passed through silica or alumina (Florisil) columns and the compound is eluted by organic solvents such as ether and/or hexane-benzene combinations. Shafik, Sullivan, and Enos (1973) concluded that silica gel column chromatography not only provided a clean sample for gas chromatographic analysis, but also conveniently separated the halogenated phenols from nitrophenols by varying the concentration of the eluting solution. Thin-layer chromatography also has been used for the purification of extracts (Kurihara and Nakajima, 1974).

B.2.6.3 Determination and Identification

Methods for detection of 2-chlorophenol and 2,4-dichlorophenol in environmental samples include colorimetry, chromatography, ultraviolet and infrared spectrophotometry, and mass spectrometry (Table B.2.1). The classic technique employed in the analysis of chlorophenol in water is the 4-aminoantipyrine colorimetric method (Ettinger, Ruchhoft, and Lishka, 1951; Faust and Aly, 1962; Rand, Greenberg, and Taras, 1976). This method is quite sensitive and easy to use, but it is nonspecific because the aminoantipyrine reacts with several isomers of chlorophenols to produce the same color. Thus, it is not suitable for distinguishing between 2-chlorophenol and 2,4-dichlorophenol contained in a sample.

Chromatographic techniques offer excellent selective separation and identification of chlorophenols. Paper chromatography was used by Loos, Roberts, and Alexander (1967) and Evans et al. (1971) to separate 2-chlorophenol and 2,4-dichlorophenol from bacterial cultures fed with 2,4-D. Evans et al. (1971) detected 2-chlorophenol and 2,4-dichlorophenol on the

TABLE B.2.1. METHODS OF DETERMINATION OF 2-CHLOROPHENOL AND 2,4-DICHLOROPHENOL IN SEVERAL SAMPLE MATERIALS

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
Aqueous solution of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol	Samples extracted with hexane and dansyl derivatives of chlorophenols separated by thin-layer chromatography	Fluorescence spectrometry of dansyl derivatives	In situ measurement of fluorescence on thin-layer chromatographic plate; confirmation analysis of derivatives by mass spectroscopy	Frei-Häusler, Frie, and Hutzinger, 1973
2,4-Dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol in natural waters	1000-ml acidified samples extracted with petroleum ether	Two-dimensional thin-layer chromatography at 1 μg/liter	No interferences found with coextractives	Zigler and Phillips, 1967
2-Chlorophenol, 2,4-dichlorophenol, and 2,3,4,5-tetrachlorophenol in mixtures of standard phenols	Phenols separated on a polyacrylate resin column	High-pressure liquid chromatography with uv detector	Elution of 21 phenols described	Fritz and Willis, 1973
2-Chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol in mixtures of standard chlorophenols		Gas-liquid chromatography	Comparable data obtained by gas-liquid chromatography and infrared spectrometry	Barry, Vasishth, and Shelton, 1962
2,4-Dichlorophenol and 2,4,5-trichlorophenol in rat urine	1- to 5-ml samples hydrolyzed with H_2SO_4 followed by extraction with ethyl ether and ethylation with diazoethane; eluted from silica gel column with benzene-hexane	Gas-liquid chromatography using electron-capture detector at levels of 0.1 mg/liter for 2,4-dichlorophenol and 0.01 ng/liter for 2,4,5-trichlorophenol	Silica gel chromatography for cleanup; no interference from impurities eluted by benzene	Shafik, Sullivan, and Enos, 1973
2,4-Dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol, and 2,3,4,5- and 2,3,4,6-tetrachlorophenol in rabbit urine	Extracted with diethyl ether; concentrated by distillation; eluted from silica gel column successively with hexane and benzene in hexane; eluate extracted with NaOH	Thermal conductivity gas-liquid chromatography	Silica gel column for cleanup; confirmation by infrared and mass spectrometry of chlorophenols and/or anisole derivatives; gas-liquid chromatography with electron-capture detector unsuitable because of tailing and nonreproducible detector response	Karapally, Saha, and Lee, 1973

(continued)

TABLE B.2.1 (continued)

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
2,4-Dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol in mouse urine	Partitioned into benzene and water; phenols determined in both phases	Thermal conductivity gas-liquid chromatography	Thin-layer chromatography used for purification of compounds; enzyme hydrolysis of water-soluble chlorophenol conjugates; confirmation by mass fragmentography	Kurihara and Nakajima, 1974
2,4-Dichlorophenol and 2,4,5-trichlorophenol in milk and cream	10-g acidified sample extracted with diethyl ether; eluted through alumina column with diethyl ether, followed by series of extractions of eluate with dilute NaOH and benzene	Gas-liquid chromatography using microcoulometric or electron-capture detector at levels of 0.05 μg/ml	Column chromatography for cleanup; NaOH or benzene extraction of eluate unnecessary for 2,4,5-trichlorophenol; recovery >80%	Bjerke et al., 1972
2,4-Dichlorophenol and 2,4,5-trichlorophenol in sheep and cattle tissues	5-g sample acid hydrolyzed with or without prior alkaline digestion; codistilled with water, followed by partitioning into methylene chloride formation of trimethylsilyl ether	Gas-liquid chromatography at levels of 0.01 to 0.02 μg/g	Distillation for cleanup; no interference of coextractives; alkaline digestion increases recovery; recovery >95%	Clark et al., 1975
2,4-Dichlorophenol in tissues of legumes, grass, and small grain plants	Homogenized in methanol; extracted with diethyl ether; eluted through alumina column; eluate extracted with NH_4OH, acidified and partitioned into benzene	Gas-liquid chromatography using electron-capture detector at levels of 0.1 μg/g	Removal of plant pigments with methylene dichloride; further cleanup on alumina column	Steen et al., 1974
2-Chlorophenol and 2,4-dichlorophenol in bacterial culture	Culture extracted with ether and steam distilled	Paper chromatography		Evans et al., 1971
2,4-Dichlorophenol in bacterial culture	Culture fed with 2,4-D and centrifuged; supernatant containing 2,4-dichlorophenol acidified and and extracted with CS_2	Gas-liquid chromatography using electron-capture detector, infrared spectrometry		Loos, Roberts, and Alexander, 1967
2,4-Dichlorophenol in bacterial culture	Culture fed with 2,4-D and centrifuged; supernatant containing 2,4-dichlorophenol acidified and steam distilled	Ultraviolet absorption spectrometry		Loos, Roberts, and Alexander, 1967

(continued)

TABLE B.2.1 (continued)

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
2,4-Dichlorophenol in bacterial culture	Formation of 2,4-dinitrophenol with $NaNO_3$	Paper chromatography		Loos, Roberts, and Alexander, 1967
2,4-Dichlorophenol in egg yolk	20-g sample blended in an ethanol, H_2SO_4, and hexane mixture; extracted with hexane-benzene	Gas-liquid chromatography using electron-capture detector at nanogram levels		Sherman, Beck, and Herrick, 1972
2,4-Dichlorophenol in hen muscle	100-g sample blended in a hexane and H_2SO_4 mixture; extracted stepwise with NaOH and hexane	Gas-liquid chromatography using electron-capture detector at nanogram levels		Sherman, Beck, and Herrick, 1972
2,4-Dichlorophenol in hen liver	25 g of liver ground and eluted with hexane; eluate extracted with CH_3CN followed by hexane	Gas-liquid chromatography using electron-capture detector at nanogram levels	Florisil column for cleanup	Sherman, Beck, and Herrick, 1972
2-Chlorophenol and 2,4-dichlorophenol in polluted water	100-ml sample steam distilled, acidified, and extracted with petroleum ether	Reaction with 4-aminoantipyrine, colorimetry; 70 μg/liter	Cleanup accomplished during distillation and extraction	Rand, Greenberg, and Taras, 1976
2-Chlorophenol and 2,4-dichlorophenol in wastewater	Sample injected directly	Gas-liquid chromatography using flame ionization detector at submilligram per liter levels	Filter to remove suspended materials which may interfere; interference from organics eliminated by distillation	Baker, 1966; Baird et al., 1974; Rand, Greenberg, and Taras, 1976
2-Chlorophenol, 2,4-dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol in water	Chlorophenols absorbed on activated carbon; adsorbates removed by stepwise extraction with $CHCl_3$, NaOH, and diethyl ether	Gas-liquid chromatography	Florisil column for cleanup	Eichelberger, Dressman, and Longbottom, 1970
2-Chlorophenol and 2,4-dichlorophenol in surface water	Chlorophenols absorbed on activated carbon; adsorbates removed by extraction with $CHCl_3$ and partitioned into acetone	Formation of pentafluorobenzyl ether derivatives followed by gas-liquid chromatography using electron-capture detector at subnanogram levels	Derivative formation eliminated cleanup step	Kawahara, 1971
2-Chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol in water	Sample used to prepare 4-aminoantipyrine and p-nitrophenylazo dye derivatives and extracted with $CHCl_3$ or ether	Thin-layer chromatography		Aly, 1968

chromatogram as the diazo dyes, and Loos, Roberts, and Alexander (1967) detected the nitro derivative of 2,4-dichlorophenol. Estimation of chlorophenols in water by thin-layer chromatography was reported (Zigler and Phillips, 1967; Aly, 1968). Zigler and Phillips (1967) separated 2,4-dichlorophenol from other chlorophenols using two-dimensional thin-layer chromatography with $AgNO_3$ and 4-aminoantipyrine as the solvents. The use of two chromogenic reagents provided a high degree of specificity. Aly (1968) formed antipyryl and *p*-nitrophenylazo dye derivatives of 2-chlorophenol and 2,4-dichlorophenol before subjecting them to separation by thin-layer chromatography. Better resolution of the two isomers was obtained with the *p*-nitrophenylazo derivative. Paper chromatography and thin-layer chromatography are simple and rapid and provide excellent resolution of chlorophenols.

Gas-liquid chromatography, the most widely used technique of separating, identifying, and quantifying chlorophenols in various kinds of samples, employs either electron-capture or flame-ionization detectors (Table B.2.1). Because gas-liquid chromatography is sensitive to nanogram levels, it is adaptable for the quantitation of trace amounts of chlorophenols. Although paper chromatography and thin-layer chromatography can resolve chlorophenols satisfactorily, they are, at best, semiquantitative methods. Gas-liquid chromatographic analysis of water and wastewater by direct aqueous injection (Baker, 1966; Baird et al., 1974) or by injection after extraction and cleanup of samples (Eichelberger, Dressman, and Longbottom, 1970; Kawahara, 1971) has been described. Direct injection of water samples simplifies gas-liquid chromatographic analysis but requires removal of interferences due to particulate matter and other organic compounds to obtain good resolution and to prolong the life of the columns. Additionally, injecting an aqueous medium may decrease the sensitivity of the detector. Kawahara (1971) successfully determined 2-chlorophenol and 2,4-dichlorophenol in surface waters in the presence of organic contaminants such as mercaptans and organic acids by electron-capture gas-liquid chromatography after forming the pentafluorobenzyl derivatives of the compounds. Cleanup was not necessary because of the selective specificity and sensitivity of the pentafluorobenzyl derivatives. 2,4-Dichlorophenol has been identified by gas-liquid chromatography as one of the major metabolites in the metabolism of 2,4-D in microorganisms (Loos, Roberts, and Alexander, 1967), animals (Bjerke et al., 1972; Clark et al., 1975), and plants (Steen et al., 1974); of lindane in animals (Karapally, Saha, and Lee, 1973; Kurihara and Nakajima, 1974); and of nemacide in hens (Sherman, Beck, and Herrick, 1972).

Biological samples such as animal and plant tissues require more extensive cleanup than water samples before they are suitable for gas-liquid chromatography. Problems of poor resolution and tailing encountered on some samples can be corrected by judicious selection of columns and type of derivatization (Kawahara, 1971; Karapally, Saha, and Lee, 1973; Shafik, Sullivan, and Enos, 1973; Baird et al., 1974). Columns reported to be satisfactory for the separation of 2-chlorophenol and 2,4-dichlorophenol include 4% dinonyl phthalate on 80/100-mesh Chromosorb G (Baird et al., 1974), 10% FFAP (free fatty acid phase) on 60/80-mesh Chromosorb T (Baker, 1966; Kawahara, 1971), 20% Carbowax 20M–terephthalic acid on 60/80-mesh

Chromosorb W-AW (Baker, 1966), 4% SE-30/6% QF-1 on 80/100-mesh Chromosorb W-HP (Shafik, Sullivan, and Enos, 1973), 1.5% neopentyl glycol succinate polyester on 60/80-mesh Chromosorb W-AW (Kurihara and Nakajima, 1974), 6% DC-200 silicone oil on 80/100-mesh Gas-Chrom Q (Bjerke et al., 1972), 15% diethylene glycol succinate/2% H_3PO_4 on 80/100-mesh Gas-Chrom P (Steen et al., 1974), 3% OV-1 on 80/90-mesh Chromosorb W (Clark et al., 1975), and 3% OV-17 on 60/80-mesh Chromosorb W-AW-DMCS (Kurihara and Nakajima, 1974). Cook and Spangelo (1974) reported the use of columns utilizing liquid crystal stationary phases for the separation of *m*- and *p*-substituted isomeric chlorophenols with similar boiling points and polarities. The ether derivative of chlorophenols formed with diazomethane or diazoethane is commonly used to improve the resolution and to confirm the identity of 2-chlorophenol and 2,4-dichlorophenol (Shafik, Sullivan, and Enos, 1973; Kurihara and Nakajima, 1974). Shafik, Sullivan, and Enos (1973) obtained better gas chromatographic resolution of more volatile chlorophenols by preparing the ethyl rather than the methyl esters. Kawahara (1971) was able to determine subnanogram amounts of 2-chlorophenol and 2,4-dichlorophenol without cleanup by reacting the compounds with α-bromo-2,3,4,5,6-pentafluorotoluene to form the pentafluorobenzyl esters. Cook and Spangelo (1974) completely separated the isomers when the chlorophenols were converted to substituted phenyl *n*-alkyl ethers.

Ultraviolet and infrared spectrophotometry have also been used to identify 2-chlorophenol and 2,4-dichlorophenol (Barry, Vasishth, and Shelton, 1962; Loos, Roberts, and Alexander, 1967). However, these methods are less sensitive than gas-liquid chromatography and are probably more useful to confirm the identity of the compounds. Mass spectrometry is also used for confirmatory analysis of chlorophenols (Frei-Häusler, Frei, and Hutzinger, 1973; Safe, Jamieson, and Hutzinger, 1974), but this technique is complex and expensive. All of these methods require prior separation of the compounds of interest before identification is made.

B.2.6.4 Assessment of Methods

Gas-liquid chromatography is the apparent method of choice for the separation, identification, and quantification of low levels of chlorophenols found in a wide variety of environmental samples. This method is rapid and sensitive and provides excellent simultaneous resolution of chlorophenol isomers. The cost of the basic instrument is probably competitive with ultraviolet and infrared instruments but is much less expensive than the mass spectrometer. The aminoantipyrine colorimetric method, although simple and rapid, lacks specificity; thus, it is only useful for gross determination of chlorophenols. Thin-layer chromatography, infrared and ultraviolet spectrophotometry, and mass spectrometry provide specific identification but require large concentrations for detection.

SECTION B.2

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B.3 BIOLOGICAL ASPECTS IN MICROORGANISMS

B.3.1 BACTERIA

B.3.1.1 Metabolism

2-Chlorophenol and 2,4-dichlorophenol are amenable to metabolism by bacteria found in soil and aquatic environments. The processes and pathways involved in metabolism are discussed fully in Section B.7; therefore, only a brief overview is presented in this section.

Spokes and Walker (1974) used phenol-adapted bacterial cultures isolated from soils to demonstrate the cometabolism of 2-chlorophenol by *Pseudomonas* sp., *Nocardia* sp., *Bacillus* sp., and *Mycobacterium coeliacum*. 2-Chlorophenol was oxidized to 3-chlorocatechol. Further breakdown of 3-chlorocatechol through ring cleavage is evident (Evans et al., 1971).

Soil bacteria, namely *Arthrobacter* sp. and *Pseudomonas* sp., capable of growing on and degrading 2,4-dichlorophenoxyacetic acid (2,4-D) can also metabolize 2-chlorophenol and 2,4-dichlorophenol (Loos, Bollag, and Alexander, 1967; Loos, Roberts, and Alexander, 1967; Bollag, Helling, and Alexander, 1968; Evans et al., 1971). The metabolism of chlorophenols by cells grown on 2,4-D, as indicated by loss of ultraviolet absorbancy and chloride release after 3 hr of incubation, is shown in Table B.3.1. Disappearance of ultraviolet absorption indicates cleavage of the aromatic ring. In studies on the fate of 2,4-dichlorophenol, Bollag et al. (1968) and Bollag, Helling, and Alexander (1968) obtained strong evidence for the transformation of the compound to 3,5-dichlorocatechol by *Arthrobacter* sp. Subsequent metabolism of the catechol resulted in cleavage of the aromatic ring to form muconic acid, which may have been converted to dicarboxylic acids, acetate, and chloride.

Aquatic bacteria, principally those present in activated sludge, have shown an ability to degrade chlorophenols (Hemmett, 1972; Nachtigall and Butler, 1974). This degradation is extremely important for the biological treatment of wastes generated from the manufacture of chlorophenols and 2,4-D. Nachtigall and Butler (1974) isolated organisms from activated sludge enriched with various phenolic compounds, including 2-chlorophenol and 2,4-dichlorophenol. Organisms identified were *Pseudomonas* sp. and *Nocardia* sp. Warburg respirometric studies indicated that *Pseudomonas* sp. oxidized all phenols and chlorophenols, but *Nocardia* sp. oxidized phenol and the monochlorophenols. *Pseudomonas* sp. isolated and grown on 2-phenylphenol oxidized the compound and cooxidized monochlorophenols and dichlorophenols. Several species of bacteria are apparently capable of using chlorophenols as their sole carbon source or are capable of utilizing the chemicals after being adapted to closely related compounds.

B.3.1.2 Effects

The bactericidal properties of chlorophenols have been known for many years (Klarmann, 1963; Sykes, 1965; Baker, Schumacher, and Roman,

TABLE B.3.1. METABOLISM OF CHLOROPHENOLS BY AN *ARTHROBACTER* SPECIES GROWN ON 2,4-D OR CITRATE

Substrate	Activity by cells grown on			
	2,4-D		Citrate	
	Loss of uv absorbancy[a]	Chloride release[b] (%)	Loss of uv absorbancy[a]	Chloride release[b] (%)
2,4-D	+ + +	96	-	0
4-Chlorophenoxyacetic acid	+ +	60	-	0
2-Chlorophenoxyacetic acid	+ +	20	-	0
6-Hydroxy-2,4-dichlorophenoxyacetic acid	-	0		
2-Hydroxy-4-chlorophenoxyacetic acid	+	2		
2,4-Dichlorophenol	+ + +	96	(+)	14
4-Chlorophenol	+ + +	98	+	10
2-Chlorophenol	+ + +	97	+	5
4-Chlorocatechol	+ + +	81	(+)	73
3,5-Dichlorocatechol	+ + +	107[c]	+	0
Catechol	+ + +		+ +	

[a] + + +, complete disappearance of the substrate absorption spectrum; + +, considerable decrease in the intensity of absorption without a change in the shape of the spectrum; +, slight decrease in the intensity of absorption without a change in the shape of the spectrum; (+), change in the shape of the spectrum to yield a poorly defined spectrum, with or without a change in the intensity of absorption; -, no change in the shape of the spectrum or in the intensity of absorption.

[b] Percentage of the total chlorine released as chloride by the end of the incubation period.

[c] When the experiment was repeated with a different batch of cells, the value was 48.

Source: Adapted from Loos, Roberts, and Alexander, 1967, Table I, p. 683. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Microbiology, Volume 13, pp. 679-690, 1967.

1970). Potentiation of antibacterial effectiveness generally increases with degree of chlorine substitution up to the trichloro derivatives (Table A.3.2). The tetrachloro isomers are considerably less active than any of the trichloro isomers, and pentachlorophenol is about as effective as the phenol itself. However, some of the higher chlorophenols have enhanced fungicidal activity, particularly pentachlorophenol.

Bactericidal action of disinfectants is usually expressed in terms of the phenol coefficient. The phenol coefficient measures, under identical conditions, the highest dilution of test disinfectants effective to kill in 10 min relative to phenol. Thus, disinfectants with high phenol coefficients are more potent. Table A.3.2 compares the antibacterial action of 2-chlorophenol and 2,4-dichlorophenol with other chlorophenols and alkyl chlorophenols on *Salmonella typhosa* and *Staphylococcus aureus*. Klarmann, Shternov, and Gates (1933) found variation in species susceptibility to 2-chlorophenol. The phenol coefficients for *Salmonella typhosa*, *Salmonella schottmuelleri*, *Staphylococcus aureas*, a homolytic strain of *Streptococcus*, *Mycobacterium smegmatis*, and *Trichophyton rosaceum* were 2.5, 2.3, 2.9, 2.0, 2.2, and <1.0 respectively.

The effect of 2,4-dichlorophenol on the growth rate of *Pseudomonas* sp., a bacterial species that abounds in soils, was studied in batch and continuous culture (Tyler and Finn, 1974). The growth rate of the organism was strongly inhibited by 2,4-dichlorophenol at concentrations above 25 mg/liter. Growth followed Monod kinetics at subinhibitory concentrations, above which the growth rate decreased linearly with concentration and did not fit a model based on noncompetitive inhibition. The lag phase of batch cultures was found to depend on 2,4-dichlorophenol concentration and prior adaptation of the inoculum.

The mode of antibacterial action of phenolic compunds may involve several steps beginning with adsorption on the bacterial cells, going through the inactivation of certain essential enzymes, and terminating with lysis and death of cells (Klarmann, 1963). The degree of cell inactivation depends largely on concentration. At higher concentrations, the chemicals act as a gross protoplasmic poison, rapidly penetrating and rupturing the cell walls; low concentrations permit the leakage of cell constituents and inactivation of specific enzyme systems. Some studies on the mechanism of action of chlorophenols have indicated that cell membrane damage is a primary effect (Judis, 1962, 1963, 1965*a*). Judis (1965*b*) investigated the effect of 2,4-dichlorophenol on the incorporation of ^{14}C-labeled substrates by *Escherichia coli*. When *E. coli* was exposed to a concentration (66.5 μg/ml) sufficient to inhibit growth in a synthetic medium, the organism was able to incorporate substantial amounts of radioactivity from several amino acids but negligible amounts from thymine, acetate, and succinate. Incorporation was completely inhibited by doubling the concentration to 133 μg/ml. Results suggest that at concentrations sufficient to prevent growth, 2,4-dichlorophenol does not inhibit synthesis of protein and ribonucleic acid but deactivates succinic dehydrogenase. Succinic dehydrogenase is known to be located in the cell membrane; therefore, any damage to the cell wall by the bactericide is detrimental to the activity of the enzyme. In addition, bacterial lipids are found mostly in the cell wall so that lipophilic compounds tend to associate with lipids and alter cell permeability (Baker, Schumacher, and Roman, 1970).

B.3.2 FUNGI

B.3.2.1 Metabolism

Few investigations of the metabolism of monochlorophenols and dichlorophenols by fungi were found; these are discussed in Section B.7. Walker (1973) demonstrated the cometabolism of 2-chlorophenol and 2,4-dichlorophenol by phenol-grown cells of the yeast *Rhodotorula glutinis*. Involvement of the induced enzyme system was manifested by the inability of the organism to utilize chlorophenols as a sole carbon source. Results indicate that the phenomenon of cometabolism of aromatic halogen compounds is not restricted to bacteria. Oxidases (laccase, tyrosinase, and peroxidase) found in white rot fungi are thought to be responsible for the degradation of phenols in wood materials. In a study of the detoxification of chlorophenols, including 2,4-dichlorophenol, Lyr (1962) concluded that these enzymes are involved in the degradation of the chlorophenols by decay fungi.

The nature of degradation products resulting from fungal metabolism has not been identified.

B.3.2.2 Effects

Chlorophenols possess fungicidal properties. The antifungal activity of 2-chlorophenol has been evaluated by testing it against *Aspergillus niger*, one of the more resistant species of fungi (Mason, Brown, and Minga, 1951; Shirk and Corey, 1952). Table B.3.2 shows the inhibiting concentration of phenolic compounds, including 2-chlorophenol, in sterilized broth solution inoculated with *A. niger* at 37°C. Although 2-chlorophenol is more effective than phenol, its potency is less than that of either 3- or 4-chlorophenol and several substituted phenolic compounds. Shirk and Corey (1952) reported that a 0.0014 *M* concentration of 2-chlorophenol inhibited growth of *A. niger* by 50% as compared with a 0.0045 *M* concentration of phenol.

TABLE B.3.2. INHIBITORY EFFECT OF PHENOLIC COMPOUNDS ON *ASPERGILLUS NIGER*

Compound	Inhibiting concentration (%)
3-Chlorophenol	0.015
4-Chlorophenol	0.015
2-Chlorophenol	0.030
Phenol	0.060
2-Chloro-4-nitrophenol	0.0075
4-Nitrophenol, sodium salt	0.025
4-Phenylphenol	0.0038
2-Chloro-4-phenylphenol, sodium salt (Dowicide D)	0.0075
Mixture of 2- and 4-chloro-6-phenylphenol, sodium salt (Dowicide 31)	0.0075
2-Bromo-4-phenylphenol, sodium salt (Dowicide 5)	0.031
2,2'-Methylenebis(4-chlorophenol)	0.0063
2,4,5-Trichlorophenol, sodium salt (Dowicide B)	0.0075
Pentachlorophenol, sodium salt (Dowicide G)	0.015
2,3,4,6-Tetrachlorophenol, sodium salt (Dowicide F)	0.031

Source: Adapted from Mason, Brown, and Minga, 1951, Table 2, p. 167. Reprinted by permission of the publisher.

B.3.3 ALGAE

B.3.3.1 Metabolism

No data on algal metabolism of monochlorophenols and dichlorophenols are available.

B.3.3.2 Effects

Algae are important in waste stabilization ponds for reoxygenation purposes. Therefore, there is considerable concern about the toxic effects of organic chemicals, including chlorophenols, on the photosynthetic activity of algae. Huang and Gloyna (1967) investigated the effects of chlorophenols and other organic chemicals on the synthesis of chlorophyll by the blue-green alga *Chlorella pyrenoidosa* under laboratory conditions. *Chlorella* was selected as the test organism because it is commonly found in streams and waste stabilization ponds; also, it is most frequently used in physiological and biochemical studies. Table A.3.4 indicates the chlorophyll content of algae treated with different levels of chlorophenols, and Table A.3.5 lists the toxicity constants. As seen in Table A.3.4, concentrations of 2-chlorophenol above 10 mg/liter progressively decreased the chlorophyll content of the algae. At 500 mg/liter, chlorophyll synthesis was completely inhibited. Smaller amounts of 2,4-dichlorophenol were needed to impair the photosynthesis of the algal cells; at 100 mg/liter the cells ceased to function. Evidently, 2,4-dichlorophenol is almost five times as toxic as 2-chlorophenol. From toxicity data, it was concluded that increasing the number of chlorine atoms on the aromatic ring of chlorophenols enhanced their toxicity. The same relationships have been reported with regard to the bactericidal properties of these chemicals.

SECTION B.3

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B.4 BIOLOGICAL ASPECTS IN PLANTS

B.4.1 METABOLISM

B.4.1.1 Uptake and Absorption

No information on the uptake of 2-chlorophenol in vascular plants was found. Based upon its chemical similarity to 2,4-dichlorophenol, residues of 2-chlorophenol may occur in plants following metabolism of 2,4-dichlorophenol.

2,4-Dichlorophenol may be absorbed by vascular plants through the roots or foliage, or it may be produced in vascular plants as a result of detoxification of the commonly used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). The formation of 2,4-dichlorophenol from 2,4-D has been demonstrated in sunflowers, corn, barley, strawberries, kidney beans, and other vascular plants (Luckwill and Lloyd-Jones, 1960; Doedens, 1963; Steen et al., 1974). The transport and distribution of 2,4-dichlorophenol formed during 2,4-D degradation is expected to be similar to that of 2,4-dichlorophenol absorbed by root or foliar uptake.

Uptake of 2,4-dichlorophenol from nutrient solutions and soils or uptake following foliar application was studied by Isensee and Jones (1971). Oat (*Avena sativa* L. 'Markton') and soybean (*Glycine max* L. 'Lee') tissues grown in nutrient solution containing ^{14}C-labeled 2,4-dichlorophenol were analyzed from 1 to 14 days following introduction of 2,4-dichlorophenol into the medium. 2,4-Dichlorophenol levels in the roots and shoots of treated soybeans and oats are shown in Table B.4.1. 2,4-Dichlorophenol reached a stable concentration in the roots within 24 hr and remained constant or declined for the remainder of the experiment. Furthermore, as much as 85% of the 2,4-dichlorophenol present in the nutrient solution was sorbed by the roots of soybeans and up to 71% was sorbed by oats. These data are difficult to interpret because the degree to which absorption or adsorption contributes to the total root content is unknown. However, substantial amounts of 2,4-dichlorophenol are clearly sorbed by the roots of oats and soybeans within 24 hr.

Absorption of 2,4-dichlorophenol from treated soil was also studied by Isensee and Jones (1971). In a greenhouse experiment, oats and soybeans were planted in pots of Lakeland sandy loam treated with ^{14}C-labeled 2,4-dichlorophenol and watered when needed. The shoots of the soybean and oat plants were harvested on the 6th, 10th, 20th, 25th, 30th, and 40th days after planting. Soybean and oat plants were also harvested on the 50th and 85th day, respectively, and the plant tissues were analyzed for 2,4-dichlorophenol (Table B.4.2). The 2,4-dichlorophenol content (microgram per gram) decreased as the age of the soybean and oat plants increased. In general, the total content of 2,4-dichlorophenol (expressed as a percentage of the total radioactivity of the soils) increased for the first 15 to 20 days and remained relatively constant thereafter. Tissue dilution, metabolism, volatilization from the tissue, or translocation back to the roots could account for a relationship of this type; however, it

TABLE B.4.1. UPTAKE OF ^{14}C-LABELED 2,4-DICHLOROPHENOL BY TEN-DAY-OLD OAT AND SOYBEAN PLANTS FROM NUTRIENT SOLUTIONS CONTAINING 0.18 TO 0.26 mg/liter 2,4-DICHLOROPHENOL

Time in nutrient solution (days)	2,4-Dichlorophenol content[a]			
	Roots		Shoots	
	(μg/g dry tissue)	(%)[b]	(μg/g dry tissue)	(%)[b]
		Soybeans		
1	273 *u*	83.6 *u*	0.33 *vw*	0.29 *w*
2	239 *uv*	83.4 *u*	0.40 *uv*	0.40 *vw*
4	178 *vw*	83.3 *u*	0.49 *u*	0.64 *u*
6	115 *vw*	75.0 *v*	0.36 *uv*	0.50 *uv*
8	132 *w*	79.3 *uv*	0.19 *wx*	0.33 *vw*
10	128 *w*	84.7 *u*	0.11 *x*	0.32 *w*
12	107 *w*	83.8 *u*	0.10 *x*	0.30 *w*
14	87 *w*	75.0 *v*	0.13 *x*	0.32 *w*
Control[c]	0.060		0.002	
		Oats		
1	87 *u*	56.0 *u*	2.05 *u*	0.46 *u*
2	100 *uv*	61.8 *vw*	2.40 *v*	0.69 *uv*
4	99 *uv*	59.8 *uv*	2.74 *u*	0.86 *uvw*
6	106 *v*	66.5 *wxy*	1.88 *w*	0.73 *uv*
8	101 *uv*	72.5 *z*	1.89 *u*	1.07 *vw*
10	98 *uv*	71.2 *yz*	1.91 *u*	1.03 *vw*
12	87 *u*	65.4 *wx*	1.73 *u*	1.02 *vw*
14	90 *u*	68.5 *xyz*	1.85 *u*	1.37 *w*
Control[c]	0.070		0.010	

[a] Numbers followed by the same letter are not significantly different at the 5% level of probability.

[b] Tissue content expressed as percentage of total activity available to plants.

[c] Plants grown in nutrient solution devoid of 2,4-dichlorophenol and harvested at 14 days.

Source: Adapted from Isensee and Jones, 1971, Table I, p. 1211. Reprinted by permission of the publisher.

TABLE B.4.2. UPTAKE OF ^{14}C-LABELED 2,4-DICHLOROPHENOL BY OATS AND SOYBEANS FROM SOIL CONTAINING 0.07 mg/kg OF 2,4-DICHLOROPHENOL

Time in nutrient solution (days)	2,4-Dichlorophenol content[a]			
	Oats		Soybeans	
	(μg/g dry tissue)	(%)[b]	(μg/g dry tissue)	(%)[b]
	Treated soil			
6	0.195 *u*	0.049	1.480 *u*	0.889 *u*
10	0.173 *u*	0.130 *v*	0.085 *v*	0.055 *x*
15	0.115 *v*	0.149 *v*	0.093 *v*	0.127 *w*
20	0.093 *vw*	0.197 *u*	0.083 *v*	0.187 *vw*
25	0.073 *wx*	0.164 *uv*	0.048 *v*	0.192 *v*
30	0.055 *xy*	0.154 *v*	0.040 *v*	0.215 *v*
40	0.038 *y*	0.160 *uv*	0.028 *v*	0.170 *vw*
50 (tissue)			0.023 *v*	0.174 *vw*
50 (seeds)			0.011	0.003
85 (tissue)	0.010 *z*	0.062 *w*		
85 (seeds)	<0.001			
	Untreated soil			
10	0.032		0.028	
20	0.010		0.006	
30	0.005		0.002	
50 (tissue)			0.003	
50 (seeds)				
85 (tissue)	<0.001			
85 (seeds)	<0.001			

[a] Numbers followed by the same letter are not significantly different at the 5% level of probability.

[b] Tissue content expressed as percentage of total activity available in treated soil.

Source: Adapted from Isensee and Jones, 1971, Table II, p. 1212. Reprinted by permission of the publisher.

was impossible to determine which, if any, of these processes were operating. Smaller quantities of 2,4-dichlorophenol were taken up from soil (Table B.4.2) than from solution (Table B.4.1). Plants harvested following 10, 15, 20, and 25 days of uptake of 2,4-dichlorophenol from soil corresponded roughly in age to plants harvested at 1, 4, 10, and 14 days in the nutrient solution experiment. When these values were compared on an equal-age basis, oats took up 8 to 25 times more 2,4-dichlorophenol from solution than from soil. The difference in the amounts taken up by soybeans from the two media was smaller (i.e., one to five times). Differences in uptake from soil and solution decreased with increased plant age. Thus, rates of 2,4-dichlorophenol uptake by the roots of vascular plants depend on factors such as species differences, age, and composition and type of growth media. Absorption of 2,4-dichlorophenol following foliar application was demonstrated by Isensee and Jones (1971), but the data provide little insight into uptake rates because the detergent Tween-80 was included in the 2,4-dichlorophenol formulation to enhance absorption.

B.4.1.2 Transport and Distribution

No information was found on the distribution and transport of 2-chlorophenol in vascular plants and very little was found on 2,4-dichlorophenol. The data in Tables B.4.1 and B.4.2 indicate that 2,4-dichlorophenol is transported to the shoots of vascular plants following root absorption (Isensee and Jones, 1971). However, only small quantities of 2,4-dichlorophenol absorbed by the roots were translocated to the shoots of soybean and oat plants (Table B.4.1.). Some differences in the translocation patterns of 2,4-dichlorophenol in soybeans and in oats are evident. 2,4-Dichlorophenol accumulated in soybean shoots for several days, declined slightly, and reached a stable concentration over the 14-day period of the experiment. On the other hand, 2,4-dichlorophenol levels in oat shoots reached a stable level within 24 hr which was maintained over the 14 days. A different pattern emerges when oats and soybeans absorb 2,4-dichlorophenol from treated soil (Table B.4.2). During the process of absorption from soil, the tissue concentration of 2,4-dichlorophenol (microgram per gram) decreased as the plants aged. However, total content of 2,4-dichlorophenol expressed as a percentage of the initial dose increased for 15 to 20 days and then remained relatively constant for the duration of the experiment. The authors suggested that tissue dilution resulting from plant growth may account for these relationships. Other possible explanations for this pattern are changes due to metabolism in the tissue, volatilization of 2,4-dichlorophenol from the tissue, or translocation back to the roots. Based on the data presented, it is impossible to establish which, if any, of these processes are operating in oats or soybeans. When the oats were fully ripened, no evidence was found of 2,4-dichlorophenol translocation to the grain. Small quantities (1% to 2% of total tissue content) of 2,4-dichlorophenol were found in soybean seeds; however, it was concluded that oats and soybeans are unable to concentrate the compound in their seeds.

In general, oats and soybeans absorbed and translocated small but substantial amounts of 2,4-dichlorophenol when they were grown in either nutrient solutions or soil containing 2,4-dichlorophenol (Isensee and

Jones, 1971). Following 14 days of uptake from a nutrient solution containing 0.20 mg/liter of 2,4-dichlorophenol, seedling oats and soybeans contained 1.84 and 0.13 μg/g of the compound respectively (i.e., a concentration factor for oats of nine times the 2,4-dichlorophenol concentration in the nutrient solution). Soybeans apparently do not concentrate 2,4-dichlorophenol from nutrient solution. After growing to maturity on soil containing 0.07 mg/kg 2,4-dichlorophenol, oats and soybeans contained 0.01 and 0.02 μg/g 2,4-dichlorophenol respectively; thus, little or no concentration of 2,4-dichlorophenol from soil was noted.

These investigations by Isensee and Jones (1971) indicate that 2,4-dichlorophenol applied to leaves is not translocated in soybeans. Soybean plants were harvested 2, 7, 14, and 21 days following foliar application of 2,4-dichlorophenol to the center leaflet of the first trifoliate leaf. The plants were dissected into six parts: treated leaflet, remainder of the first trifoliate leaf plus petiole, second trifoliate, all remaining trifoliate leaves, first true leaves, and stem. These samples were analyzed individually for 2,4-dichlorophenol; the data indicated that 2,4-dichlorophenol was not translocated beyond the treated leaflet. Carbon-14 activity detected in the untreated portions of the plants was equal to or less than the background activity for control plants. Because no visual phytotoxicity was evident from the application of 2,4-dichlorophenol, it was assumed that the capacity for translocation was not impaired. Forty-eight hours after application, 2% of the applied 2,4-dichlorophenol remained on the treated soybean leaf. Volatilization from the leaf surface was believed to account for this loss; application of 2,4-dichlorophenol to glass surfaces resulted in 96% volatilization in 48 hr.

B.4.1.3 Biotransformation

The metabolism of 2-chlorophenol in vascular plants is not well studied. The only report found demonstrated that 2-chlorophenol may be inactivated by glycoside formation in plant tissue. When certain nonnaturally occurring chemicals are absorbed by various plants, glycoside formation takes place with the foreign chemical serving as the aglycon. Miller (1941) found that the metabolic fate of 2-chlorophenol in tomato plants included glycoside formation. Tomato plants of the Marglobe and Bonny Best varieties were grown in sand cultures containing 2-chlorophenol. Tomato plants (3 to 4 in. high) were planted in sand cultures, and 2-chlorophenol was added when the plants were 13 to 14 in. high (approximately 33 days after transfer to the sand culture). The 2-chlorophenol was added four to six times per week, and each pot received 0.1 m*M* 2-chlorophenol at each application point. A total of 2.8 m*M* 2-chlorophenol was added to each culture in "5-inch pots." The pots probably had a capacity of about 3000 g soil to give a total addition of 100 to 120 mg chlorophenol per kilogram soil. β-*o*-Chlorophenyl gentiobioside (a glycoside of 2-chlorophenol) was isolated from the roots of these tomato plants. There was no evidence that the glycoside was formed in shoots. The fate of this metabolic product of 2-chlorophenol in plants is not known and warrants further investigation. Glycoside formation likely involves the immobilization of the toxic chemical, thereby preventing it from reaching the normal sites of its toxic action.

Information on the biotransformation of 2,4-dichlorophenol is also scant. Considerable information does exist, however, on the production of 2,4-dichlorophenol as a metabolic breakdown product of other compounds in vascular plants. Some highlights of these studies are presented because this information directly relates to possible sources of 2,4-dichlorophenol in plants.

Investigations conducted by Luckwill and Lloyd-Jones (1960) indicate that 2,4-dichlorophenol may be detoxified in the strawberry by conjugation, specifically by glycoside formation and immobilization of the compound. Experiments showed that quantitative recovery of 2,4-dichlorophenol from Tallisman strawberry leaves could only be achieved following hydrolysis of the plant tissue with NaOH under reflux conditions. No information was given on the enzymes or enzyme systems responsible for the conjugation, the types of degradation products formed, or the rate of transformation. Chkanikov et al. (1976) reported that the high resistance of strawberries to 2,4-D is caused by destruction of the ether bond in the herbicide, leading to the formation of 2,4-dichlorophenol. Other means of detoxification such as ring cleavage of 2,4-dichlorophenol are possible; however, no data addressing this question are available.

Bristol et al. (1974) determined the persistence times of 2,4-D and 2,4-dichlorophenol in potato tubers. Analyses of potato tubers for 2,4-D and 2,4-dichlorophenol showed a residue level of 2,4-dichlorophenol which was greater than 10% of the 2,4-D residue level. Dissipation rates of 2,4-D and 2,4-dichlorophenol from whole, treated potatoes stored at 38°F (3°C) were determined; 2,4-D residues decreased with a half-life of about 12 weeks, but residues of 2,4-dichlorophenol remained constant over this period.

Sokolov et al. (1974) studied the metabolism, sorption, migration, and persistence of 2,4-D in irrigated rice fields. Large quantities of 2,4-dichlorophenol were found in rice plants and in soil following herbicidal application. Although 2,4-D residues were found in rice plants during the first month of application, rice grains contained neither herbicide nor metabolite (2,4-dichlorophenol) residues when the plants reached maturity.

B.4.1.4 Elimination

Investigations on the route or rate of 2-chlorophenol and 2,4-dichlorophenol elimination from vascular plants were not found. Unfortunately, the uptake studies conducted by Isensee and Jones (1971) do not allow calculation of excretion rates or routes. 2,4-Dichlorophenol is likely dissipated from vascular plants by one of three routes: volatilization from leaf surfaces or stems, metabolism of the compound with subsequent excretion of metabolic breakdown products, or excretion through the roots. Isensee and Jones (1971) demonstrated that >98% of the 2,4-dichlorophenol applied to the foliage of soybean plants was lost, probably due to volatilization, within 72 hr of application. Information regarding metabolic breakdown of 2,4-dichlorophenol and subsequent excretion of metabolic products is not available. Dexter, Slife, and Butler (1971) found that certain plants avoid injury following exposure to 2,4-D by excretion through their root systems. Thus, a precedent exists for possible excretion by the roots as a route of 2,4-dichlorophenol elimination from plants; however, no studies addressed this point.

B.4.2 EFFECTS

2-Chlorophenol or 2,4-dichlorophenol would likely be applied to soil or plants only if they were combined with or were contaminants of some other material. Neither is used in any manner involving direct application to plants. The herbicide 2,4-D may be contaminated with 2,4-dichlorophenol, but no data are available on the extent of contamination. The most likely source of these chlorophenols in plants is degradation of plant-assimilated 2,4-D.

B.4.2.1 Physiological or Biochemical Role

There is no evidence that 2-chlorophenol or 2,4-dichlorophenol play any necessary metabolic role in vascular plants; thus, their presence in plant tissues probably results from environmental contamination.

B.4.2.2 Toxicity

B.4.2.2.1 Mechanism of Action - Very little information on the mechanisms of toxicity of 2-chlorophenol or 2,4-dichlorophenol to vascular plants was found. Some notable effects of 2-chlorophenol and 2,4-dichlorophenol on in vitro enzyme systems have been documented.

2-Chlorophenol produced reversible inhibition of etiolated pea brei (a finely divided tissue suspension) catalase and of crystalline beef liver catalase in vitro at a level of 4×10^{-5} *M*. The importance of the phenomenon is unknown (Goldacre and Galston, 1953).

A number of reports have documented an effect of 2,4-dichlorophenol on in vitro enzyme systems. Indoleacetic acid is a phytohormone which is important in the growth regulation of plants. Indoleacetic acid oxidase is an enzyme system which regulates endogenous indoleacetic acid levels and presumably plays a role in growth regulation. It has been found that the in vitro activity of indoleacetic acid oxidase enzyme preparations depends on the presence of 2,4-dichlorophenol (or related phenols). This phenomenon has been observed in indoleacetic acid oxidase systems derived from the pea (*Pisum sativum*) epicotyl (Taniguchi, Yamaguchi, and Satomura, 1973), root extracts of *Lens culinaris* and *Phaseolus vulgaris* (Dinant, Gaspar, and Avella, 1967), and maize (*Zea mays*) root preparations (Psenakova et al., 1971). The mechanism for this cofactor-type activity of 2,4-dichlorophenol is not understood. Because 2,4-dichlorophenol has not been found as a normal constituent of plant tissue, it must be assumed that by virtue of its chemical structure it mimics compounds which normally act on the indoleacetic acid oxidase system in plants. Goldacre and Galston (1953) found that 2,4-dichlorophenol produced a reversible in vitro inhibition of etiolated pea brei catalase and of crystalline beef liver catalase. Other heme enzymes (e.g., peroxidase, cytochrome oxidase, and hemoglobin) were unaffected by 2,4-dichlorophenol at a concentration of 2×10^{-6} M. The bactericidal properties of 2,4-dichlorophenol may be due in part to this effect on the catalase system. Furthermore, the fact that 2,4-dichlorophenol inhibits catalase without affecting peroxidase indicates that 2,4-dichlorophenol may block catalase activity in reactions where catalase may be expected to compete with peroxidase for available H_2O_2. This phenomenon may partly account for the 2,4-dichlorophenol activation of indoleacetic acid oxidase mentioned previously.

B.4.2.2.2 General Toxicity — No information exists on the phytotoxic effects of 2-chlorophenol in vascular plants. Due to the chemical similarity to 2,4-D, 2,4-dichlorophenol may display some of the same toxic effects in plants as the herbicide; however, data on the toxicity of 2,4-dichlorophenol to vascular plants are scant.

2,4-Dichlorophenol inhibited root growth in flax seedlings, cell expansion in wheat coleoptiles, and the growth-stimulating effect of indoleacetic acid in wheat (Volynets and Pal'chenko, 1972). Luckwill and Lloyd-Jones (1960) found that apples, strawberries, and currants appeared to possess a detoxification mechanism for 2,4-dichlorophenol. Relatively large quantities of 2,4-dichlorophenol were fed through the transpiration stream of leaves of these plants, but no phytotoxic symptoms were observed. These plant varieties are also resistant to the effects of 2,4-D. Because 2,4-D may be decarboxylated to yield 2,4-dichlorophenol in vascular plants, the detoxification mechanism responsible for 2,4-dichlorophenol inactivation may be a portion of an overall 2,4-D metabolizing system. No information was found on the toxicological properties of 2,4-dichlorophenol in terrestrial vascular plants, but one report discussed the toxicological effects of 2,4-dichlorophenol on the aquatic plant *Lemna minor*. Blackman et al. (1955*a*) determined the 2,4-dichlorophenol concentration in water causing the death of 50% of the *Lemna minor* fronds following a 48-hr exposure; the LC_{50} value was 40 mg/liter. Other investigations by Blackman et al. (1955*b*) indicate that the physiological effect of chlorophenols is increased as the pH of the medium approaches the p*K* of the chlorophenol.

The metabolism of chlorophenoxyacetic acids to chlorophenols occurs in many plant species. A typical investigation demonstrating the production of 2,4-dichlorophenol from 2,4-D was conducted by Steen et al. (1974). 2,4-Dichlorophenol levels in plant tissues after foliar and root application of 2,4-D are given in Tables B.4.3 and B.4.4.

B.4.2.2.3 Mitotic Effects — No information regarding possible mitotic effects of 2-chlorophenol was found, but several studies reported on the effect of 2,4-dichlorophenol on plant mitosis. Amer and Ali (1969) studied the effect of 2,4-dichlorophenol on root mitosis of *Vicia faba*. At a concentration of 62.5 mg/liter 2,4-dichlorophenol, there was a decreased mitotic index and an increased frequency of induced mitotic anomalies, such as cytomixis, stickiness, lagging chromosomes, formation of anaphase bridges, and fragmentation (Table B.4.5).

Further investigations on the mitotic effect of 2,4-dichlorophenol on *Vicia faba* were later conducted by Amer and Ali (1974). Fifteen- or

TABLE B.4.3. LEVELS OF 2,4-DICHLOROPHENOL AND 2,4-D IN AERIAL PORTIONS OF PLANTS AFTER TREATMENT WITH 2,4-D

Plant	Residue levels (μg/g fresh wt)[a]	
	2,4-Dichlorophenol	2,4-D
Kidney bean (*Phaseolus vulgaris*)	0.4	60.1
Soybean (*Glycine max*)	<0.1	18.5
Pea (*Pisum sativum*)	0.2	31.4
Bromegrass (*Bromus* sp.)	0.2	13.7
Wild oat (*Avena fatua*)	0.3	33.3
Yellow foxtail (*Setaria glauca*)	0.1	35.4
Barley (*Hordeum vulgare*)	0.4	34.5
Timothy (*Phleum pratense*)	0.5	40.6
Orchard grass (*Dactylis glomerata*)	0.2	38.7

[a] Determined 72 hr after treatment with 2,4-D in 50% acetone at a rate of 1.0 kg/ha.

Source: Adapted from Steen et al., 1974, Table 1, p. 26. Reprinted by permission of the publisher.

TABLE B.4.4. LEVELS OF 2,4-DICHLOROPHENOL AND 2,4-D IN PLANTS WHOSE ROOTS WERE TREATED WITH 2,4-D

Plant	Residue levels (μg/g fresh wt)[a]	
	2,4-Dichlorophenol	2,4-D
Kidney bean (*Phaseolus vulgaris*)	1.3	75.0
Soybean (*Glycine max*)	0.3	61.0
Pea (*Pisum sativum*)	0.4	70.0
Bromegrass (*Bromus* sp.)	0.4	86.8
Wild oat (*Avena fatua*)	0.7	70.0
Yellow foxtail (*Setaria glauca*)	0.4	89.0
Barley (*Hordeum vulgare*)	1.0	70.0
Timothy (*Phleum pratense*)	1.6	83.0
Orchard grass (*Dactylis glomerata*)	0.3	57.0

[a] Roots treated with 2,4-D at a rate of 0.1 mg ml^{-1} g^{-1} fresh wt.

Source: Adapted from Steen et al., 1974, Table 3, p. 26. Reprinted by permission of the publisher.

TABLE B.4.5. EFFECT OF 2,4-DICHLOROPHENOL ON MITOTIC ABNORMALITIES IN ROOT PREPARATIONS OF *VICIA FABA*

Cell type and mitotic stage	Control	Treated with 62.5 mg/liter 2,4-dichlorophenol
Number of dividing cells	920	1005
Number of abnormal cells	4	39
Abnormal cells, %[a]	0.43	3.88
Cells in prophase, %	55.5	68.6
Cells in metaphase, %	18.4	21.3
Abnormal cells in metaphase, %[a]	1.18	10.7
Cells in anatelophase, %	26.1	10.1
Abnormal cells in anatelophase, %[a]	0.83	15.7
Mitotic index	147	95

[a] Determined as ratio of abnormal cells to normal cells x 100.

Source: Adapted from Amer and Ali, 1969, Table 1, p. 534. Reprinted by permission of the publisher.

35-day-old plants were sprayed daily with 7 ml of aqueous 2,4-dichlorophenol solution containing 0.39 mg 2,4-dichlorophenol per liter. Flower buds were gathered when the plants were 40 days old and pollen mother cells were checked for mitotic abnormalities. Abnormalities were detected, and the effect depended on plant age. Stickiness, lagging chromosomes, and fragmentation were the most common abnormalities observed. A higher percentage of abnormal pollen mother cells was seen in 35-day-old plants than in 15-day-old plants. *Vicia faba* seeds were soaked in water containing 0.39 mg/liter of 2,4-dichlorophenol for 24 hr and planted. Analyses of pollen mother cells were performed in a manner similar to that described for sprayed plants (Table B.4.6). Significant increases in the percentage of abnormal pollen mother cells were noted when 35-day-old plants were sprayed or when seeds were soaked in a solution of 2,4-dichlorophenol. No significant increases in the percentage of abnormal pollen mother cells were noted when 15-day-old plants were sprayed. The possibility of 2,4-dichlorophenol causing pollen grain sterility was also investigated, but no adverse effects were noted. Effects on yields of the European broad bean (*Vicia faba*) were also studied (Amer and Ali, 1974). Only plants sprayed at 15 days showed a significant yield decrease. The number of pods per plant and the weight of seeds per plant decreased over control plants, which were significant at the 5% level of probability. When later generations of plants were examined, it was found that the second generation of these treated plants showed a reduced seed weight per plant, but the

TABLE B.4.6. PERCENTAGE OF ABNORMAL POLLEN MOTHER CELLS AFTER TREATMENT OF *VICIA FABA* WITH 2,4-DICHLOROPHENOL

Treatment	Number of plants	Abnormal pollen mother cells (mean %)
Seeds soaked in water	5	0.27
15- or 35-day-old plants sprayed with water	7	0.68
Seeds soaked for 24 hr in solution containing 0.39 mg/liter 2,4-dichlorophenol	8	2.24[a]
15-day-old plants sprayed daily for five days with 7-ml solution containing 0.39 mg/liter 2,4-dichlorophenol	8	1.24
35-day-old plants sprayed daily for five days with 7-ml solution containing 0.39 mg/liter 2,4-dichlorophenol	6	4.79

[a] Determination made 40 days after planting; values different from control at the 5% level of probability.

Source: Adapted from Amer and Ali, 1974, Table 1, p. 634. Reprinted by permission of the publisher.

number of pods per plant did not differ from the controls. No effect was found in the third generation.

Two distinguishable effects on mitoses of 2,4-dichlorophenol-treated pea seedlings (*Pisum sativum*) were observed by Mühling et al. (1960). The primary roots of the seedlings were suspended for 8 to 12 hr at 22.5°C in a nutrient bath containing 2,4-dichlorophenol. The plants were removed and the roots were analyzed for cytological effects. At 2,4-dichlorophenol concentrations greater than 60 mg/liter, spindle formation was inhibited, resulting in chromosome configurations very similar to those produced by the mitotic inhibitor colchicine. Also, the onset of cell division was inhibited.

SECTION B.4

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B.5 BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

B.5.1 BIOLOGICAL ASPECTS IN BIRDS AND MAMMALS

No information was found on the metabolism or physiological effects of 2-chlorophenol or 2,4-dichlorophenol in birds or mammals which were directly exposed to either compound. However, a limited number of reports discuss the appearance of 2,4-dichlorophenol in the tissues of mammals following exposure to related compounds such as 2,4-dichlorophenoxyacetic acid (2,4-D). Because most of these studies were conducted with experimental animals under laboratory conditions, the data are examined in Section B.6. Reports on the formation of 2,4-dichlorophenol by 2,4-D or nemacide degradation in domestic animals are included in Section B.5.1.1.2.

B.5.1.1 Metabolism

B.5.1.1.1 Uptake and Absorption - No reports were found on the uptake and absorption of 2-chlorophenol or 2,4-dichlorophenol in birds or mammals.

B.5.1.1.2 Transport and Distribution - No information is available on the transport and distribution of 2-chlorophenol in birds or mammals. The tissue distribution of 2,4-dichlorophenol in sheep and cattle fed 2,4-D was determined by Clark et al. (1975). The 2,4-D was administered in feed at levels of 300, 1000, and 2000 mg/kg feed. On the basis that each animal injested a daily ration of 3% of its body weight, these levels were equivalent to 9, 30, and 60 mg/kg body weight daily respectively. The animals were maintained on a diet containing 2,4-D for 28 days, and tissue samples were analyzed for 2,4-D and 2,4-dichlorophenol residues at the end of this period (Table B.5.1). 2,4-Dichlorophenol was not detected in muscle and fat; however, liver and kidney tissues contained substantial levels of 2,4-dichlorophenol. Withdrawal from 2,4-D treatment for one week before the sheep were killed resulted in a significant reduction in the residue level in the kidney, but the liver retained most of the 2,4-dichlorophenol which had accumulated. Because 2,4-D is probably detoxified in the liver following administration, high levels of 2,4-dichlorophenol are not surprising. It is difficult, however, to draw any conclusions from these data regarding the fate of 2,4-dichlorophenol administered directly to domestic animals. The authors noted that sheep and cattle ingesting 2,4-D generally ate less feed, gained weight more slowly, and had less efficient feed conversion ratios than control animals; however, it is not known whether the 2,4-dichlorophenol was responsible for the general weakening of condition. The presence of 2,4-dichlorophenol in the animals may have resulted from hydrolysis of 2,4-D by rumen microorganisms or by enzymatic processes within the animal, but no conclusive evidence for either of these pathways was presented. Bjerke et al. (1972) looked for 2,4-dichlorophenol in the milk and cream from cows dosed with 2,4-D, but no 2,4-dichlorophenol was detected when the cows were fed diets containing as much as 1000 mg 2,4-dichlorophenol per kilogram feed for two or three weeks.

TABLE B.5.1. RESIDUES OF 2,4-D AND 2,4-DICHLOROPHENOL IN SHEEP AND CATTLE FED 2,4-D FOR 28 DAYS

Tissue	Animal	Daily dose of 2,4-D (mg/kg body wt)	Residues found (mg/kg tissue)	
			2,4-D	2,4-Dichlorophenol
Muscle	Sheep	60	0.06	<0.05
		60[a]	<0.05	<0.05
	Cattle	9	<0.05	<0.05
		30	<0.05	<0.05
		60	0.07	<0.05
Fat	Sheep	60	0.10	<0.05
		60[a]	0.15	<0.05
	Cattle	9	0.13	<0.05
		30	0.45	<0.05
		60	0.34	<0.05
Liver	Sheep	60	0.98	0.16
		60[a]	0.29	0.15
	Cattle	9	<0.05	0.11
		30	0.14	0.59
		60	0.23	0.31
Kidney	Sheep	60	9.17	0.26
		60[a]	0.37	0.07
	Cattle	9	2.53	0.56
		30	8.67	1.17
		60	10.9	1.06

[a] Animals dosed with 2,4-D for 28 days and withdrawn from 2,4-D exposure for seven days before sampling.

Source: Adapted from Clark et al., 1975, Table III, p. 576. Reprinted by permission of the publisher.

Sherman, Beck, and Herrick (1972) studied the production of 2,4-dichlorophenol in laying hens following administration of nemacide [*O*-(2,4-dichlorophenyl)*O*,*O*-diethyl phosphorothioate] administered at levels of 50, 100, 200, and 800 mg/kg feed over a 55-week period. The animals were then returned to a normal diet and tissues were analyzed for 2,4-dichlorophenol residues after 5, 7, 10, 14, and 21 days. No residues of 2,4-dichlorophenol were found in the fat or breast muscle of hens treated with nemacide. However, 2,4-dichlorophenol was found in the liver and egg yolk of these hens (Table B.5.2) at levels of <0.12 to 0.61 ppm. Decreasing concentrations of 2,4-dichlorophenol in the liver were noted in animals fed lower doses of nemacide. 2,4-Dichlorophenol was detected in the livers

TABLE B.5.2. RESIDUES OF 2,4-DICHLOROPHENOL IN LAYING HENS FED DIETS CONTAINING NEMACIDE [*O*-2,4-DICHLOROPHENYL)*O*,*O*-DIETHYL PHOSPHOROTHIOATE] FOR 55 WEEKS

Nemacide in feed (mg/kg)	Time after exposure[a] (days)	2,4-Dichlorophenol residues in tissues (mg/kg)			
		Liver		Egg yolk	
		Mean	Range	Mean	Range
800	0	0.47	0.14-0.68	0.61	0.34-0.75
	5			0.27	
	7	0.50	0.25-0.75		
	10			<0.12	
	14	0.27	0.056-0.48		
	21	0.19	0.11-0.27		
200	0	0.38	0.31-0.44	0.15	0.12-0.17
	5			0.13	
	7	0.30	0.24-0.35		
	10			<0.12	
	14	0.14	0.099-0.18		
	21	0.36	0.071-0.64		
100	0	0.26	0.25-0.26	0.15	<0.12-0.21
	5			<0.12	
	7	0.56			
	10			<0.12	
	14	0.14	<0.052-0.33		
	21	<0.052			
50	0	0.31	0.16-0.46	<0.12	
	5			<0.12	
	7	0.18	0.14-0.22		
	10			<0.12	
	14	0.055	<0.052-0.11		
	21	<0.052			

[a] Hens returned to nemacide-free diets at 55 weeks and 2,4-dichlorophenol residues determined 0 to 21 days thereafter.

Source: Adapted from Sherman, Beck, and Herrick, 1972, Table VIII, p. 623. Reprinted by permission of the publisher.

for as long as 21 days following removal from treated feed in hens treated with high nemacide levels. The liver levels increased only from 0.31 ppm to 0.47 ppm (increase of 34%) on day 0, even though there was a 16-fold increase in dose (50 to 800 mg/kg).

B.5.1.1.3 Biotransformation — No reports were found on the biotransformation of 2-chlorophenol or 2,4-dichlorophenol in birds or mammals.

B.5.1.1.4 Elimination — No information was found on the elimination of 2-chlorophenol or 2,4-dichlorophenol in birds or mammals.

B.5.1.2 Effects

No studies were found on the effects of 2-chlorophenol or 2,4-dichlorophenol in birds or mammals.

B.5.2 BIOLOGICAL ASPECTS IN FISH AND OTHER AQUATIC ORGANISMS

B.5.2.1 Metabolism

B.5.2.1.1 Uptake and Absorption — Apparently, no data exist on the uptake and absorption of 2-chlorophenol and 2,4-dichlorophenol in aquatic organisms. However, from toxicological information (Section B.5.2.2), it is assumed that aquatic organisms can assimilate these chlorophenols. The route and mechanisms of entry and the factors which control rate and extent of uptake by aquatic organisms have not been determined.

B.5.2.1.2 Transport and Distribution — No investigations decribing the transport mechanism or the mode of distribution of 2-chlorophenol or 2,4-dichlorophenol in aquatic organisms were located.

B.5.2.1.3 Biotransformation — No information was found to delineate the rate or pathway of metabolism of 2-chlorophenol or 2,4-dichlorophenol in aquatic organisms.

B.5.2.1.4 Elimination — No data on the elimination of 2-chlorophenol or 2,4-dichlorophenol from aquatic organisms were found.

B.5.2.2 Effects

B.5.2.2.1 Physiological or Biochemical Role — No evidence suggests that 2-chlorophenol and 2,4-dichlorophenol perform any normal physiological function in aquatic organisms.

B.5.2.2.2 Toxicity

B.5.2.2.2.1 Mechanism of action — The biochemical basis for the toxicity of 2-chlorophenol to aquatic organisms is unknown. Mitsuda, Murakami, and Kawai (1963) reported that 2-chlorophenol inhibited oxidative phosphorylation in rat liver mitochondrial preparations, but 40 times greater concentrations of the compound were required to inhibit oxidative phosphorylation to the same extent as that found with the potent uncoupler

2,4-dinitrophenol. Inhibition of oxidative phosphorylation in rat liver mitochondria has also been demonstrated for 2,4-dichlorophenol (Mitsuda, Murakami, and Kawai, 1963). It is a much more effective uncoupler than 2-chlorophenol (see Section B.6 for more details).

B.5.2.2.2.2 General toxicity — Toxicity data for 2-chlorophenol in aquatic organisms are sparse. The minimum lethal dose for injection into frogs has been reported as 0.4 mg/kg body weight (von Oettingen, 1949). Lammering and Burbank (1960) studied the effect of 2-chlorophenol on the bluegill (*Lepomis macrochirus*) and established median tolerance limits (TL_m) — the concentration of 2-chlorophenol in the water that killed 50% of the test fish in a specified time — of 8.2 mg/liter at 24 hr and 8.1 mg/liter at 48 hr. The authors stated, "Inasmuch as the 24 and 48 hr TL_m values were nearly equivalent, it appears that prolonged exposure to *o*-chlorophenol will show little, if any, effects that are attributable to cumulative toxicity." Such short-term studies do not provide sufficient data to warrant this conclusion. Gross flesh discoloration was observed in bluegills which were exposed to 2-chlorophenol concentrations greater than 11.5 mg/liter. Physiological responses of bluegill and trout included rapid and uncoordinated movement, loss of equilibrium, nervous twitching, and an extremely excitable state in which slight movements near the aquaria would cause the fish to swim in a violent fashion.

The TL_m for 2-chlorophenol in bluegill and rainbow trout (*Salmo gairdnerii irideus*) was also determined by Sletten and Burbank (1972) (Table B.5.3). Trout were approximately three times as sensitive as bluegill to the effects of 2-chlorophenol. Sletten and Burbank (1972) developed an in vitro method for determining the effect of a toxic substance on fish tissue. The basis of this technique was to evaluate the effect

TABLE B.5.3. MEDIAN TOLERANCE LIMITS
OF BLUEGILL (*LEPOMIS MACROCHIRUS*)
AND TROUT (*SALMO GAIRDNERII*)
TO 2-CHLOROPHENOL

Fish species	Length of exposure (hr)	TL_m (mg/liter)
Bluegill	24	8.2
	48	8.1
Trout	24	2.8
	48	2.7
	96	2.6

Source: Adapted from Sletten and Burbank, 1972, Table II, p. 28. Reprinted by permission of the publisher.

of chemicals on the rate of oxygen utilization by fish tissue in a Warburg respirometer. Oxygen uptake values for tissues exposed to 2-chlorophenol were measured in 3-hr runs. The respiratory TL_m in the manometric assay was defined as that amount of 2-chlorophenol which caused a 50% reduction in oxygen uptake by the treated tissue when compared with the untreated tissue. The respiratory TL_m values for the in vitro assay on various trout tissues are shown in Table B.5.4. The authors suggested that the in vitro test may serve as a viable alternative to the more time-consuming and difficult toxicity tests currently used. Kopperman, Carlson, and Caple (1974) determined the 48-hr TL_m value for 2-chlorophenol in *Daphnia magna* to be 7.4 mg/liter.

TABLE B.5.4. RESPIRATORY MEDIAN TOLERANCE LIMITS OF TROUT TISSUES TO 2-CHLOROPHENOL[a]

Tissue	Respiratory TL_m (mg/g tissue)
Brain	0.8
Liver	0.9
Gill	3.2

[a] The concentration of 2-chlorophenol which caused a 50% reduction in oxygen uptake of the tissue compared with control tissue in a 3-hr run.

Source: Adapted from Sletten, and Burbank, 1972, Table V, p. 30. Reprinted by permission of the publisher.

Only two reports described the toxic effect of 2,4-dichlorophenol on aquatic organisms. Clowes (1951) reported that 2,4-dichlorophenol affected oxygen consumption and cell division in fertilized sea urchin eggs (*Arbacia punctulata*). At a concentration of 16.3 mg/liter, 2,4-dichlorophenol-treated sea urchin eggs consumed approximately twice as much oxygen as control eggs, and this phenomenon was accompanied by a dramatic increase in the rate of cell division. At a limiting concentration of 10.3 mg/liter, a decreased rate of cell division was initiated in treated eggs; at 163 mg/liter, cell division ceased entirely. The 48-hr TL_m of 2,4-dichlorophenol to *Daphnia magna* was reported as 2.6 mg/liter (Kopperman, Carlson, and Caple, 1974).

SECTION B.5

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B.6 BIOLOGICAL ASPECTS IN HUMANS

B.6.1 METABOLISM

B.6.1.1 Uptake and Absorption

No data on the routes or rates of entry of 2-chlorophenol in humans were found. Administration of 2-chlorophenol to experimental animals subcutaneously, intravenously, intraperitoneally, or orally may result in death (Section B.6.2), indicating that 2-chlorophenol is absorbed by the organism. It is not known if 2-chlorophenol is absorbed by the respiratory route. According to Doedens (1963), the monochlorophenols are corrosive to the skin and eyes and are absorbed readily through the skin in toxic amounts. Furthermore, the vapors or dusts are reportedly irritating and toxic. 2-Chlorophenol was not specifically discussed; therefore, its cutaneous toxicity and respiratory toxicity remain questionable.

Data on the uptake and absorption of 2,4-dichlorophenol in humans are also sparse. Toxicity to experimental animals has been demonstrated when the compound is administered by the oral, intraperitoneal, or subcutaneous routes, indicating absorption into the body by these routes. Doedens (1963) stated that the dichlorophenols may be somewhat less toxic than the monochlorophenols and are absorbed less quickly through the skin. No data directly addressing this question are available.

B.6.1.2 Transport and Distribution

No information on the transport or tissue distribution of 2-chlorophenol or 2,4-dichlorophenol was found.

B.6.1.3 Biotransformation

Data on the metabolism of 2-chlorophenol in humans are not available. Information obtained from experimental animals indicates that 2-chlorophenol is excreted primarily in the urine in both free and conjugated forms. In dogs, 87% of administered 2-chlorophenol was excreted as sulfuric and glucuronic acid conjugates (von Oettingen, 1949). The urine from these animals darkened upon standing, which indicates the formation of polyphenols. Free 2-chlorophenol, as well as the sulfate and glucuronide conjugates of the compound, was detected in rabbit urine after oral administration of monochlorobenzene (Lindsay Smith, Shaw, and Foulkes, 1972). Unfortunately, the data presented by these investigators do not allow the calculation of the relative levels of free 2-chlorophenol and its conjugates. Most of the 2-chlorophenol resulting from chlorobenzene metabolism, however, was excreted in the conjugated form.

The convulsive action of 2-chlorophenol in mice was studied by Angel and Rogers (1972). Following intraperitoneal administration of 2-chlorophenol, a rapid onset of convulsions was noted. Furthermore, a simple exponential decay of the convulsive effect indicated that removal from the central nervous system may occur by a simple chemical reaction. The authors suggested that ortho methylation in the body may be the relevant detoxification mechanism; however, no information directly addressing this point is available.

No information on the biotransformation of 2,4-dichlorophenol in humans is available, nor were reports found on the biotransformation of 2,4-dichlorophenol following administration of the compound directly to experimental animals. One investigation suggested a detoxification mechanism for 2,4-dichlorophenol in mice following intraperitoneal injection of 1,2,3,4,5,6-hexachlorocyclohexane (Kurihara and Nakajima, 1974). Carbon-14-labeled hexachlorocyclohexane isomers (γ and β) were administered to mice, and the appearance of metabolites in the urine was monitored. 2,4-Dichlorophenol and 2,4-dichlorophenol conjugates (sulfate and glucuronide) were major metabolites of γ- and β-hexachlorocyclohexane in the mouse. Free 2,4-dichlorophenol was found in the urine to a lesser extent (Table B.6.1). The data indicate that following the administration of γ-hexachlorocyclohexane, the glucuronide conjugate of 2,4-dichlorophenol was present to the greatest degree (4% to 5% of the administered hexachlorocvlohexane radioactivity). The sulfate conjugate comprised 1% of the administered hexachlorocyclohexane radioactivity. After administration of β-hexachlorocyclohexane, glucuronide and sulfate conjugates were present in the urine at approximately equal levels. If one assumes that the metabolism of 2,4-dichlorophenol which results from prior metabolism of another organic compound parallels the transformation of 2,4-dichlorophenol which is directly administered to mammals, conjugation to sulfate or glucuronide appears to be a major metabolic pathway.

TABLE B.6.1. ABUNDANCE OF THE PRINCIPAL URINARY 2,4-DICHLOROPHENOL-CONTAINING METABOLITES FROM HEXACHLOROCYCLOHEXANE ISOMERS IN THE MOUSE

Isomer administered	Abundance of metabolites in urine (% of total metabolites)			
	2,4-Dichlorophenol glucuronide	2,4-Dichlorophenol sulfate	Total 2,4-dichlorophenol conjugates	Free chlorophenols
γ-Hexachlorocyclohexane	4-5	1	4-6	3
β-Hexachlorocyclohexane	1-2	3	4-5	11[a]

[a] Free acidic compounds; individual compounds not identified.

Source: Adapted from Kurihara, 1975, Table 13, p. 68. Reprinted by permission of the publisher.

B.6.1.4 Elimination

No data on the elimination of 2-chlorophenol directly administered to humans or mammals have been found. One study which sheds some light on the excretion of 2-chlorophenol was reported by Lindsay Smith, Shaw, and Foulkes (1972). Following administration of chlorobenzene to rabbits, 2-chlorophenol appeared in the urine in both free and conjugated forms. Unfortunately, it is not possible to calculate the relative amounts of free and conjugate forms of 2-chlorophenol. Excretion primarily through the urine appears to be a likely possibility. Following administration of 2-chlorophenol to dogs, 87% of the compound was excreted in conjugation with sulfuric and glucuronic acids (von Oettingen, 1949). The urine samples on standing became darker in color, indicating the formation of polyphenols following excretion.

Elimination of 2,4-dichlorophenol from mammals can only be inferred from data on the excretion of 2,4-dichlorophenol following degradation of other compounds. After administration of lindane, Karapally, Saha, and Lee (1973) noted that 4.4% of total ether-soluble metabolites in rabbit urine was 2,4-dichlorophenol; the data do not allow calculation of urinary excretion rates. Similarly, administration of nemacide [*O*-(2,4-dichlorophenyl)*O*,*O*-diethyl phosphorothioate] to rats caused the appearance of 2,4-dichlorophenol in urine (Shafik, Sullivan, and Enos, 1973). Nemacide in peanut oil was administered to rats by gavage (feeding with a stomach tube) once a day for three days. The 2,4-dichlorophenol content of the urine was determined until the compound no longer appeared. Following administration of 1.6 mg of nemacide, 67% of the compound appeared in the urine as 2,4-dichlorophenol within three days. When rats were dosed with smaller amounts of nemacide (0.16 mg), 70% of the administered dose appeared in the urine as 2,4-dichlorophenol in one day. Apparently, excretion of 2,4-dichlorophenol was completed during these periods.

Although investigations providing information on the appearance of 2-chlorophenol or 2,4-dichlorophenol in mammals as a result of metabolism of other organic compounds were conducted primarily to understand the mechanisms of detoxification of foreign compounds, they also offer information on an alternative source of exposure to chlorophenols by mammals. The extent of this exposure is speculative; compounds other than those described may be transformed to 2-chlorophenol or 2,4-dichlorophenol in mammals, including humans. As discussed above, 2-chlorophenol was detected as a minor metabolite of chlorobenzene metabolism in rabbits (Lindsay Smith, Shaw, and Foulkes, 1972). Another investigation (Selander, Jerina, and Daly, 1975) demonstrated that chlorobenzene was converted to a mixture of 2-, 3-, and 4-monochlorophenol in perfused rat liver preparations. Three different monochlorophenols were produced, and evidence showed that three enzyme systems catalyzed the hydroxylation of chlorobenzene to monochlorophenols. 2,4-Dichlorophenol has been detected as a metabolite of γ- and β-hexachlorocyclohexane in mice, lindane (not less than 99% of the γ isomer of hexachlorocyclohexane) in the rabbit, and nemacide in the rat (Karapally, Saha, and Lee, 1973; Shafik, Sullivan, and Enos, 1973; Kurihara and Nakajima, 1974). Exposure of animals or humans to these compounds is more likely than direct exposure to 2-chlorophenol or 2,4-dichlorophenol. Neither 2-chlorophenol nor 2,4-dichlorophenol is widely used, and exposure is probably limited to workers in the manufacturing industry. Exposure to compounds such as lindane and nemacide is more likely because they have been widely used as pesticides.

B.6.2 EFFECTS

B.6.2.1 Physiological or Biochemical Role

Humans do not appear to have a normal physiological requirement for 2-chlorophenol. Similarly, no physiological requirement for 2,4-dichlorophenol in mammalian systems has been demonstrated. However, it has been shown that the enzymatic oxidation of NADH in various tissues is stimulated by the presence of 2,4-dichlorophenol (Coleman and Bever, 1968). This catalytic requirement for in vitro enzyme assay systems is relatively nonspecific; apparently, the presence of an aromatic hydroxy group is all that is required. The importance of this phenomenon in the in vitro situation is not known.

B.6.2.2 Toxicity

B.6.2.2.1 Mechanism of Action — The mechanism of toxic action of 2-chlorophenol is not well characterized. 2-Chlorophenol appears to uncouple oxidative phosphorylation in rat liver mitochondria (Mitsuda, Murakami, and Kawai, 1963); however, the effect is relatively weak. Mitsuda, Murakami, and Kawai (1963) noted that 2-chlorophenol was the weakest uncoupler of nine chlorophenols tested. In addition, Parker (1958) noted that 2-chlorophenol appeared to uncouple oxidative phosphorylation, but during the course of its action the uptake of oxygen was greatly inhibited in rat liver mitochondrial preparations. This effect contrasts with that of the strong uncouplers 2,4-dinitrophenol and pentachlorophenol; these compounds uncouple oxidative phosphorylation without a concomitant decrease in oxygen uptake. Farquharson, Gage, and Northover (1958) studied the biological action of a series of chlorinated phenols and noted that toxicity increased with increased chlorination; the convulsant action of phenol was replaced by the poisoning characteristic of dinitrophenol. Tremors and convulsions in rats were reported by Farquharson, Gage, and Northover (1958) after 2-chlorophenol was injected intraperitoneally. Tremors appeared in 40 to 120 sec. When the tremors increased in severity, intermittent convulsions developed and the rat usually fell on its side with loss of righting reflexes.

Farquharson, Gage, and Northover (1958) also examined the effect of 2-chlorophenol on the oxygen uptake of rat brain homogenates. 2-Chlorophenol caused no stimulation of oxygen uptake at concentrations ranging from 10^{-6} to 10^{-3} *M*. Angel and Rogers (1972) studied the convulsive activity of a series of organic compounds, including 2-chlorophenol. The compounds were administered intraperitoneally to anesthetized mice. 2-Chlorophenol caused myoclonic convulsions (i.e., alternate contraction and relaxation of groups of muscles). Dosages of the compounds needed to cause convulsions in 50% of the treated animals were 0.77 millimole/kg (99 mg/kg) for 2-chlorophenol and 1.04 millimoles/kg (98 mg/kg) for phenol. Thus, 2-chlorophenol was 1.3 to 1.4 times more potent than phenol, which has a well-documented convulsive action in mammals. The biochemical mechanism for this action is unknown.

No evidence suggests that 2,4-dichlorophenol has a convulsive effect on mammals, but various enzymes as well as the process of oxidative phosphorylation have been affected. Stockdale and Selwyn (1971) demonstrated that 2,4-dichlorophenol inhibited the activity of lactate dehydrogenase and hexokinase in an in vitro system. These investigators suggested that this inhibition resulted from the binding of 2,4-dichlorophenol to the enzyme at a site which normally binds the adenine group in the substrate. The physiological significance of this enzyme inhibition is not known. 2,4-Dichlorophenol also has inhibited oxidative phosphorylation in rat liver mitochondria and rat brain homogenate preparations (Farquharson, Gage, and Northover, 1958; Parker, 1958; Mitsuda, Murakami, and Kawai, 1963). According to Farquharson, Gage, and Northover (1958), oxygen uptake in rat brain homogenates peaks (70% above control values) at a 2,4-dichlorophenol concentration of 2.5×10^{-4} *M*. Mitsuda, Murakami, and Kawai (1963) calculated that a concentration of 4.2×10^{-5} *M* 2,4-dichlorophenol inhibited oxidative phosphorylation by 50% in rat liver mitochondrial preparations; this value was roughly intermediate among the nine chlorophenols tested. The inhibition was approximately twofold weaker than that of 2,4-dinitrophenol, a strong uncoupler of oxidative phosphorylation. It is not clear how this effect on oxidative phosphorylation contributes to the toxicity of 2,4-dichlorophenol in mammals, but it seems likely that it may be responsible for the toxicity of the compound.

B.6.2.2.2 General Toxicity — No information exists on the toxicity of 2-chlorophenol to humans, but toxicity has been documented in experimental animals. Lethal dosages for various experimental animals are compiled in Table A.6.4. Signs of 2-chlorophenol intoxication in rats are similar whether the compound is administered subcutaneously, intraperitoneally, or orally. The toxicological picture includes restlessness and increased rate of respiration within a few minutes following administration. Somewhat later motor weakness develops and tremors and convulsions induced by noise or touch occur. Eventually, dyspnea and coma appear and continue until death (Farquharson, Gage, and Northover, 1958). Following fatal poisoning, the pathology of the rat includes marked kidney injury, the presence of red blood cell casts in the tubules, fatty infiltration in the liver, and hemorrhages in the intestine (Deichmann and Keplinger, 1963). Bubnov, Yaphizov, and Ogryzkov (1969) reported a similar pathological picture in the blue fox and the mouse. Lethal concentrations of 2-chlorophenol caused fatty degeneration of the liver, renal granular dystrophy, and necrosis of the stomach and intestinal mucosa.

The toxicology of 2,4-dichlorophenol is poorly documented. It is apparently moderately toxic to animals (Sax, 1975). Only a few LD_{50} values for 2,4-dichlorophenol in experimental animals have been published (Table A.6.4). A comprehensive study of the chronic toxicity of 2,4-dichlorophenol in mice was conducted by Kobayashi et al. (1972). Male mice were fed a diet containing the compound for six months. Various parameters, including average body weight, food consumption, organ weight, and histological findings in major organs, were measured throughout the treatment period. No adverse changes in the behavior, growth rate, or blood glutamic oxaloacetate and glutamic or pyruvate

TABLE B.6.2. HISTOLOGICAL CHANGES IN MAJOR ORGANS OF MALE MICE (SEVEN ANIMALS PER GROUP) FED 2,4-DICHLOROPHENOL DAILY FOR SIX MONTHS

Organ	Observation	Number of changes		
		Control	100 mg/kg 2,4-dichlorophenol	230 mg/kg 2,4-dichlorophenol
Heart	Localized degenerative changes in myocardium	1	0	1
Liver	Small round cell infiltration	0	0	2
	Swelling of hepatic cell	0	0	1
	Unequal size of hepatic cell	0	0	1
	Dark cell	1	0	2
	Focal necrosis	1	0	0
	Interstitial reaction	0	2	0
Kidneys	Small round cell infiltration	4	3	3
	Collapse of glomerulus	0	1	1
	Interstitial cell infiltration	1	0	0
Spleen	Atrophic change in lymphatic layer of white pulps	0	0	1
Adrenal glands	Thinner cortex	0	0	2

Source: Adapted from Kobayashi et al., 1972, Table 8, p. 360. Reprinted by permission of the publisher.

transaminase levels were found in mice receiving up to a maximum daily dose of 230 mg/kg. Minor histological changes occurred in the liver of animals receiving this dose (Table B.6.2). The authors concluded that 2,4-dichlorophenol is a safe substance and set a daily dose of 100 mg/kg as a maximum no-effect level in mice.

Only one report suggested that 2,4-dichlorophenol may have adverse effects in humans. Bleiberg et al. (1964) reported 29 cases of acquired chloracne and 11 cases of porphyria in workers involved in the manufacture of 2,4-dichlorophenol and 2,4,5-trichlorophenol. The patients were exposed to a group of basic chemicals including acetic acid, phenol, monochloroacetic acid, sodium hydroxide, 2,4-dichlorophenol, and 2,4,5-trichlorophenol in the occupational environment. The agents responsible for the symptoms cannot be determined clearly, nor can the degree of involvement of 2,4-dichlorophenol in skin pathology and liver damage be assessed (Table B.6.3).

TABLE B.6.3. SUMMARY OF DATA FOR 26 WORKERS INVOLVED IN MANUFACTURE OF 2,4-DICHLOROPHENOL AND 2,4,5-TRICHLOROPHENOL

Patient	Chloracne	Hyperpigmentation	Hirsutism	Urine uroporphyrins	Chemical contact[b]	Skin fragility
1	Severe	Mild	Moderate	Positive	Moderate	Positive
2	Mild	None	None	None	Severe	Negative
3	Mild	Mild	None	None	Severe	Negative
4[c]	Severe	Mild	Mild	None	Severe	Positive
5	Mild	None	Mild	Positive	Severe	Positive
6	Mild	None	None	None	Moderate	Negative
7	Mild	Mild	None	Positive	Moderate	Negative
8	Severe	Moderate	Severe	None	Moderate	Negative
9	Mild	Mild	Mild	None	Moderate	Negative
10	Moderate	Moderate	Moderate	None	Severe	Negative
11	Severe	Moderate	Moderate	None	Moderate	Negative
12	None	None	None	None	Severe	Negative
13	None	None	None	None	Severe	Negative
14	None	None	None	Positive	Moderate	Negative
15	Moderate	Mild	Moderate	None	Moderate	Positive
16	Moderate	Moderate	Marked	None	Severe	Positive
17	Mild	Mild	None	None	Mild	Negative
18	Moderate	Moderate	Marked	None	Mild	Negative
19	Severe	Marked	None	Positive	Mild	Negative
20	Mild	Mild	Moderate	Positive	Mild	Negative
21	None	None	None	None	Mild	Negative
22	None	None	None	None	Mild	Negative
23	Mild	None	None	None	Mild	Negative
24	None	None	None	None	Mild	Negative
25	None	None	None	Positive	Mild	Negative
26	None	None	None	Positive	Mild	Negative

[a] Severity of chloracne judged on presence of comedones, epidermoid cysts, and furuncles and pustules.

[b] Extent of exposure difficult to judge because of variables such as personal hygiene and work habits.

[c] Brief period of employment.

Source: Adapted from Bleiberg et al., 1964, p. 795. Reprinted by permission of the publisher.

B.6.2.2.3 Carcinogenicity — Repeated applications of phenol and some substituted phenols reportedly can promote the appearance of skin tumors in mice following a single initiating dose of dimethylbenzanthracene (Boutwell and Bosch, 1959). Tumors also developed in mice (not exposed to dimethylbenzanthracene) treated with phenol alone for long periods. When 2-chlorophenol and 2,4-dichlorophenol were tested for tumorigenicity and tumor-promoting activity in a similar system (Boutwell and Bosch, 1959), a tumor-promoting activity was present. No evidence exists that either 2-chlorophenol or 2,4-dichlorophenol may cause tumors in the absence of an initiator (i.e., dimethylbenzanthracene). Both 2-chlorophenol and 2,4-dichlorophenol possessed approximately the same activity in promoting tumor formation as phenol (Table B.6.4). Both benign and malignant tumors were formed. The hazardous effect to humans exposed to either of these compounds is not known. Protracted skin contact is not recommended because of the potential for tumor formation and because both compounds are primary skin irritants.

TABLE B.6.4. APPEARANCE OF SKIN TUMORS IN MICE TREATED CUTANEOUSLY WITH PHENOLS FOLLOWING A CUTANEOUS DOSE OF 0.3% DIMETHYLBENZANTHRACENE IN ACETONE

Treatment[a]	Time animals examined (weeks)	Number of mice (survivors/total)	Survivors with papilloma (%)	Survivors with epithelial carcinoma (%)
Benzene control	12	12/12	0	0
10% phenol in benzene, no dimethylbenzanthracene	20	24/30	33	13
20% phenol in acetone	12	21/24	58	5
20% phenol in benzene	24	10/33	100	20
20% 2-chlorophenol in benzene	15	31/35	61	10
20% 2,4-dichlorophenol in benzene	15	27/33	48	11

[a] All received dimethylbenzanthracene except where stated.

Source: Adapted from Boutwell and Bosch, 1959, Table 2, pp. 418–420. Reprinted by permission of the publisher.

SECTION B.6

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B.7 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

B.7.1 TRENDS IN PRODUCTION AND USE

Production trends for 2-chlorophenol and 2,4-dichlorophenol are difficult to assess because data reported to the U.S. International Trade Commission (U.S. International Trade Commission, 1976) by manufacturers are regarded as proprietary. Dow Chemical Company produces both chemicals; the Monsanto Company manufactures only 2,4-dichlorophenol. 2,4-Dichlorophenol production in the United States can be estimated based on the manufacture of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) (Table B.7.1). Data indicate declining production of the herbicide from 1967 to 1971.

TABLE B.7.1. PRODUCTION OF 2,4-D COMPOUNDS IN THE UNITED STATES, 1967-1975

Year	Production (metric tons)		
	2,4-D	2,4-D esters and salts	Total
1967	35,021	3001	38,022
1968	35,985	6743	42,728
1969	21,373	4504	25,877
1970	19,784	*b*	*b*
1971	*b*	*b*	24,943[c]
1972	*b*	*b*	*b*
1973	*b*	*b*	*b*
1974	*b*	*b*	*b*
1975	*b*	*b*	*b*

[a] Withheld to avoid disclosure.

[b] Based on 15,714,000 kg used by farmers, which represents 63% of the total usage; excludes exports but includes imports.

Source: Compiled from U.S. Department of Agriculture, 1976.

Important uses which relate to the possible distribution of these chlorophenols in the environment are discussed in this section. More detailed discussion of general uses is found in Section B.2. 2-Chlorophenol is used principally as an intermediate for the manufacture of higher chlorophenols (Dow Chemical Company, 1977). Its use as a constituent of fire retardants has been supplanted by polybrominated biphenyl.

The direct use of this chemical as a bactericide or germicide is probably negligible because more effective compounds, such as higher chlorophenols, have been utilized. The single most important use of 2,4-dichlorophenol is in the manufacture of pesticides, particularly 2,4-D. The widespread application of 2,4-D and its derivatives for the control of weeds is likely the main course by which dichlorophenol finds its way into the environment. Although 2,4-dichlorophenol has germicidal properties, its use as such may be negligible.

B.7.2 SOURCES OF POLLUTION

B.7.2.1 Distribution in Air

Reports on the sources and distribution of chlorophenols in the atmosphere are not available. Because of the lack of atmospheric monitoring data for these compounds, their sources, both point and nonpoint, are only speculative. Potential for large discharges of chlorophenols into the atmosphere exists in plants where these compounds and hormone-type pesticides are manufactured. The volatilization of these chemicals from water, soil, foliage, and impervious surfaces may play an important role in their dispersal to the atmosphere, but no quantitative data are available. Furthermore, it might be speculated that incineration could generate volatile products to the atmosphere through the burning of containers and trash containing chlorophenols.

B.7.2.2 Distribution in Aquatic Environments

Industrial waste discharge is the principal point source of water pollution. During the manufacture of chlorophenols and 2,4-D a considerable amount of chemical waste is generated as a result of incomplete reaction of the starting reactants, by-product formation, and incomplete recovery of desired products. Thus, the wastes contain a mixture of chlorophenols and other compounds. Waste arising from the manufacture of phenoxyalkanoic herbicides showed amounts of 2-chlorophenol ranging from a trace to 6% and of 2,4-dichlorophenol ranging from 11% to 89% (Sidwell, 1971) (Tables B.7.2 and B.7.3).

Other possible point sources are chemical spills and washing of containers or drums in which chlorophenols and 2,4-D are stored. Contamination of water with 2-chlorophenol and 2,4-dichlorophenol may arise from (1) chlorination of phenol present in natural water and primary and secondary effluents of waste treatment plants (Burttschell et al., 1959; Eisenhauer, 1964; Manufacturing Chemists Association, 1972), (2) direct addition of the chemicals or their presence as contaminants or degradation products of 2,4-D used for aquatic weed control, and (3) wet and dry atmospheric fallout.

Runoff from urban and agricultural watersheds could be an important nonpoint source of chlorophenols in aquatic environments. For this to occur, the primary sources are likely to be contaminants or degradation products of 2,4-D compounds which are applied widely on croplands, lawns, and golf courses for the control of weeds. The contribution of runoff

TABLE B.7.2. ANALYSIS OF INDUSTRIAL PLANT WASTE FROM HERCULES, INC., JACKSONVILLE, ARKANSAS, 1970

Parameter	Sampling date				
	January 25	March 3	April 21	May 28	August 27
Temperature, °C	12	18	21	28.5	24
pH	7.5	7.6	7.4	7.4	7.0
Total alkalinity to pH 4.2, mg/liter	560	2,250	3,960	4,510	305
Biochemical oxygen demand, mg/liter	515	1,680	3,840	6,315	400
Chemical oxygen demand, mg/liter	700	2,500	6,200	8,315	1,290
Total solids, mg/liter	6,960	40,100	76,320	104,860	11,000
Suspended solids, mg/liter	160	360	380	580	Nil
Settleable solids, mg/liter	6	16	40	40	1.3
Chloride, mg/liter	3,000	19,350	37,350	52,150	4,950
Chlorophenols, mg/liter	68	118	125	112	74
Phenoxy acids, mg/liter	167	183	241	235	199
Volume, liters	37,600	360,600	114,500	78,100	5,400
Dissolved oxygen, mg/liter	6	5.8	3.0	[a]	[a]
Weather	Clear	Heavy rain	Clear	Clear	Clear

[a] No data.

Source: Adapted from Sidwell, 1971, Table XXI, p. 51.

TABLE B.7.3. RELATIVE CHLOROPHENOL CONTENT OF INDUSTRIAL WASTE FROM HERCULES, INC., JACKSONVILLE, ARKANSAS, 1970

Compound	Chlorophenol content (% of total phenols)				
	January 25	March 3	April 21	May 28	August 27
Phenol	3.4	6.2	1.7	24.8	Trace
2-Chlorophenol	2.9	6.1	Trace	Trace	Trace
4-Chlorophenol	2.5	12.1	18.3	20.0	2.8
2,4-Dichlorophenol	73.6	17.9	20.0	11.4	89.0
2,5-Dichlorophenol	Trace	6.2	1.7	Trace	1.8
2,6-Dichlorophenol	9.9	41.7	38.8	30.5	3.0
2,4,5-Trichlorophenol	4.7	Trace	Trace	Trace	Trace
2,4,6-Trichlorophenol	2.8	9.9	19.5	13.3	3.4

Source: Adapted from Sidwell, 1971, Table XXI-A, p. 51.

to the levels of chlorophenols in water has yet to be documented. There is no information on the distribution of 2-chlorophenol and 2,4-dichlorophenol in water, aquatic organisms, and sediments.

B.7.2.3 Distribution in Soil

The primary source of soil contamination by chlorophenols is probably through the application of 2,4-D and its derivatives on croplands. In 1971, U.S. farmers applied almost 16,000,000 kg of 2,4-D, representing 15% of all organic herbicide usage (U.S. Department of Agriculture, 1974). Although usage declined slightly compared with 1966 usage, there most likely has been substantial use over the past five years. Commercial formulations of 2,4-D reportedly contain 2,4-dichlorophenol as an impurity; the amount ranges from 70 to 4500 mg/kg (Aly and Faust, 1965). In addition, 2,4-D is degraded rapidly by soil microorganisms and produces 2,4-dichlorophenol as an early metabolite. Therefore, the 2,4-dichlorophenol generated from herbicide applications has the potential to accumulate, depending on environmental conditions, and to move, depending on its interactions with the soil.

The major factors affecting the distribution of 2,4-dichlorophenol in soils are degradation and sorption. If the chemical persists due to environmental conditions unfavorable for microbial growth, then it has more time to interact with the soil. Movement of 2,4-dichlorophenol in soil has apparently not been investigated. Evidence shows that the compound is weakly sorbed, making it susceptible to leaching through the soil profile. However, at normal rates of application the herbicide and its metabolites are degraded readily so that downward movement is minimized.

Groundwater contamination with 2,4-D and 2,4-dichlorophenol is evident when large doses are released into the environment. In 1945, a company began to manufacture 2,4-D in California, emptying wastes containing 2,4-D and 2,4-dichlorophenol into the local sewage system. Within 17 days, phenolic tastes and odors were reported downstream in shallow wells, and they persisted for three years (Swenson, 1962). At the Rocky Mountain Arsenal in Colorado, 2,4-D was manufactured and wastes were discharged into lagoons from 1943 to 1957. Groundwater contamination was first reported in 1951 when crops were damaged by irrigation well water (Walker, 1961). It took seven to eight years for the contaminant to migrate 5.6 km and to affect an area of 16.8 km^2.

B.7.3 ENVIRONMENTAL FATE

B.7.3.1 Mobility and Persistence in Air

The forms in which 2-chlorophenol and 2,4-dichlorophenol are transported in the atmosphere are unknown. Although these compounds are capable of being dispersed to the atmosphere through volatilization, no investigations have been made on their presence, movement, fate, and persistence. Possibly, photodecomposition is an important mechanism of dissipation.

B.7.3.2 Mobility and Persistence in Aquatic Environments

Chlorophenols may be present in the aquatic environment as the dissolved form, associated with suspended matter or bottom sediments, and absorbed in biological tissues. Hydrological factors such as pattern of currents and mixing and decay and migration of organisms affect the movement of these chemicals. However, no data have been reported to support this premise.

A few studies indicate that the dissipation of 2-chlorophenol in water is microbiologically mediated. Ettinger and Ruchhoft (1950) investigated the persistence of monochlorophenols in polluted river water and diluted sewage at 20°C. Low concentrations (1 mg/liter) of 2-chlorophenol added to a usual dilution of domestic sewage were not removed during periods of 20 to 30 days, presumably due to the absence of microorganisms capable of attacking the chemical. When a similar concentration was added to polluted river waters, the compound dissipated in 15 to 23 days (Table B.7.4). Addition of a seed consisting of water from a previous persistence experiment significantly increased the removal of 2-chlorophenol. Apparently, the seed introduced some organisms already adapted to the chemical. This study also indicates that the removal of monochlorophenols requires the presence of a specialized microflora.

TABLE B.7.4. PERSISTENCE OF MONOCHLOROPHENOLS ADDED TO SEVERAL SURFACE WATERS AT 20°C

Time stored (days)	Residual monochlorophenols (μg/liter)			
	Great Miami River water		Little Miami River water	
	2-Chlorophenol	4-Chlorophenol	2-Chlorophenol	4-Chlorophenol
0	980	1000	890	1000
6	810	980	870	1000
13	790	80	480	0
15	760	0	0	0
23	710	0	0	0
36	0	0	0	0

Source: Adapted from Ettinger and Ruchhoft, 1950, Table 2, p. 3c.

Baird et al. (1974) employed Warburg respiratory techniques to demonstrate that biodegradation of 2-chlorophenol at a concentration of 1 mg/liter in activated sludge was complete within 3 hr. Increasing the concentration to 100 mg/liter reduced considerably the rate of respiration so that only 20% was degraded in 6 hr. In a sludge not acclimated with high levels of 2-chlorophenol, certain amounts of the compound may be degraded initially, and oxidative intermediates that appear subsequently could be toxic to the microbial population. This study indicates that 2-chlorophenol may persist longer if waste containing high levels of the chemical is discharged to an unacclimated body of water due to direct or indirect toxic effects.

The persistence of 2,4-dichlorophenol in the aquatic environment appears to be short. Aly and Faust (1964) studied the disappearance of 2,4-dichlorophenol at concentrations of 100, 500, and 1000 μg/liter in natural lake waters buffered at pH 7.0 and continually aerated. Complete disappearance of 2,4-dichlorophenol occurred within 9 days from the 100 μg/liter treatment, and 97.5% dissipated from the 500 and 1000 μg/liter treatments in 30 days (Table B.7.5). At all rates of application, 50% of the chlorophenol was decomposed in a matter of 6 days. To simulate the effect of excessive amounts of decaying organic matter on biological decomposition, ethanolic solutions of 2,4-dichlorophenol were added to unbuffered, unaerated lake water. 2,4-Dichlorophenol persisted for more than 43 days. Anaerobic conditions, as indicated by the presence of

TABLE B.7.5. PERSISTENCE OF 2,4-DICHLOROPHENOL IN LAKE WATER AFTER ADDITIONS OF 100, 500, OR 1000 μg/liter 2,4-DICHLOROPHENOL

Time (days)	100 μg/liter		500 μg/liter		1000 μg/liter	
	pH	Oxidation (%)	pH	Oxidation (%)	pH	Oxidation (%)
			Aerated and buffered			
0	7.4	0.0	7.4	0.0	7.4	0.0
2	7.3	36.0	7.6	22.0	7.6	24.0
9	7.3	100.0	7.5	66.0	7.4	54.0
16	6.9	100.0	7.1	81.6	7.2	83.5
23	*a*	*a*	7.5	93.6	7.5	92.2
30	*a*	*a*	7.3	97.5	7.3	97.5
			Unaerated and unbuffered			
0	7.3	0.0	7.3	0.0	7.3	0.0
3	6.2	20.0	5.1	22.0	4.1	22.0
7	6.1	30.0	6.1	24.0	6.0	23.0
14	*a*	*a*	*a*	*a*	6.1	38.0
17	7.9	60.0	6.5	49.4	6.3	44.0
24	*a*	60.0	*a*	61.6	*a*	46.0
43	*a*	80.0	*a*	61.6	*a*	49.0

[a] No data.

Source: Adapted from Aly and Faust, 1964, Table VII, p. 546. Reprinted by permission of the publisher.

hydrogen sulfide, prevailed in the system. This limited information suggests the importance of biological degradation in 2,4-dichlorophenol dissipation. Although the compound appears to be easily degraded, limnological factors such as oxygen depletion could inhibit the process and thereby extend its persistence. Similar observations were noted for 2,4-D. The effects of anaerobic and aerobic conditions on 2,4-D, as modified by temperature, were determined in a sediment-free impoundment model with controlled chemical and thermal stratification (Demarco, Symons, and Robeck, 1967).

Sidwell (1971) studied the persistence of 2,4-dichlorophenol in aeration lagoon effluent. The initial concentrations of 2,4-dichlorophenol and 2,4-D were 64 and 174 mg/liter respectively. Constant aeration was introduced to the acid-phenol effluent, which was adjusted to pH 7.0 and maintained at a temperature of 20°C to 21°C. 2,4-Dichlorophenol disappeared within five days — more rapidly than 2,4-D (Figure B.7.1). This removal rate indicates that sufficient nutrients and a large microbial population capable of metabolizing the chlorophenol were present in the effluent.

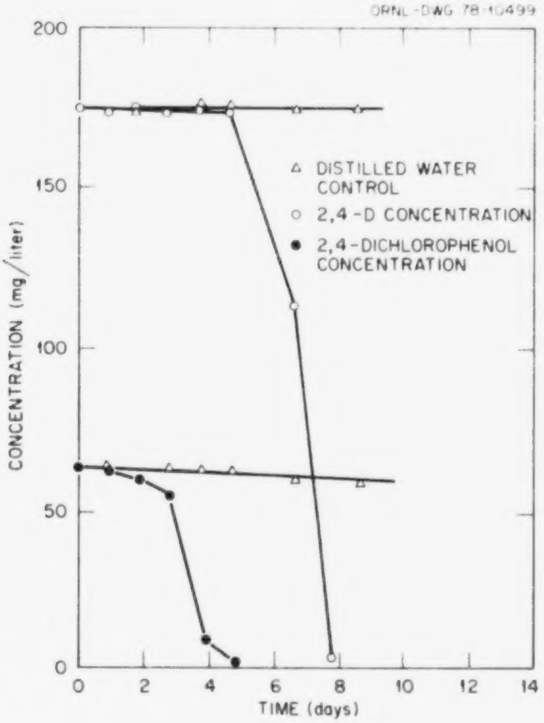

Figure B.7.1. Removal of 2,4-dichlorophenol and 2,4-D from solution in aeration basin effluent by continuous aeration. Source: Adapted from Sidwell, 1971, Figure 5, p. 67.

Conditions affecting the biodegradability of chlorophenols were investigated by Ingols, Gaffney, and Stevenson (1966) with acclimated, activated sludge. The effects of pH, temperature, and concentration are given in Tables B.7.6 and B.7.7. Optimum temperature for degradation of 2-chlorophenol appeared to be between 25°C and 27°C, and optimum pH ranged from 6.5 to 8.0. Degradation was uninhibited by up to 100 mg/liter of 2,4-dichlorophenol. Complete ring cleavage of 2-chlorophenol and 2,4-dichlorophenol occurred in three and five days respectively (Table A.7.1).

B.7.3.3 Mobility and Persistence in Soil

The most important interaction of chlorophenols in soils is sorption. The extent of sorption determines whether the compounds are carried in association with eroded soils during overland flow or are leached through the soil profile during infiltration. Soil factors affecting sorption are pH, moisture, and content of clay and organic matter.

Sorption of 2-chlorophenol by soils has not been studied, but 2,4-dichlorophenol sorption by clay minerals has been reported. Aly and Faust (1964) found that 2,4-dichlorophenol and 2,4-D compounds were sorbed by bentonite, illite, and kaolinite, but the magnitude was small as shown by the high amount of clays required to remove significant amounts of each compound from an aqueous suspension (Table B.7.8). 2,4-Dichlorophenol was sorbed by illite and bentonite to a greater extent than was the sodium salt of 2,4-D. Extent of sorption was directly proportional to the specific surface area of the clay minerals. Thus, bentonite, an expanding-lattice clay mineral, had the highest sorption followed by the nonexpanding-lattice clays, illite and kaolinite. Because 2,4-dichlorophenol is a weak acid, it will ionize to varying degrees depending on the pH of the system. Negative sorption of organic anions — formed as a result of ionization of acidic pesticides — by clay colloids occurred at pH values in excess of 7, but positive sorption of molecular species occurred in strongly acidic systems (Weber, 1972). Although sorption of molecular species through physical forces was probably the primary mechanism, sorption of anionic species may also occur because kaolinite, illite, and hydrous metallic oxides possess anion-exchange properties.

Organic matter is a soil component largely responsible for the sorption of many organic compounds. Information on 2,4-dichlorophenol sorption by organic matter is not available; therefore, its sorption by this component can only be inferred from studies on acidic pesticides such as 2,4-D. Acidic pesticides were sorbed to organic soil colloids which were controlled by the pH of the system; sorption was greater under acidic conditions where the pesticides were sorbed in the molecular form (Hamaker, Goring, and Youngson, 1966). The amounts of acidic pesticides sorbed were much lower than amounts of pesticide groups such as the s-triazines, organochlorine, and bipyridyl compounds (Weber, 1972). Sorption probably occurred through hydrogen bonding or physical sorption of molecular species to the organic colloidal surfaces. Sorbed species were easily desorbed by water, indicating that the sorption bond strength is

TABLE B.7.6. EFFECT OF TEMPERATURE AND pH ON THE DEGRADATION OF 2-CHLOROPHENOL[a]

pH	Degradation (%)			
	20°C	25°C	27°C	30°C
6.5	33	*b*	88	*b*
6.8	*b*	74	*b*	57
7.3	27	*b*	89	*b*
7.9	20	*b*	91	*b*
8.4	*b*	22	*b*	6
9.3	*b*	3	*b*	0

[a] 200 mg/liter concentration; acclimated activated sludge aerated for one day.

[b] No data.

Source: Adapted from Ingols, Gaffney, and Stevenson, Table 1, p. 631. Reprinted by permission of the publisher.

TABLE B.7.7. EFFECT OF CONCENTRATION ON THE DEGRADATION OF 2,4-DICHLOROPHENOL[a]

Compound	Concentration (mg/liter)	Ring cleavage (%)	Development of chloride ion (%)
2,4-Dichlorophenol, after two days	50	80	47
	100	75	35
	200	40	12
	400	55	3
2,4,6-Trichlorophenol, after two days	50	100	19
	100	100	28
	200	60	5
	400	0	0
Dichloroquinone, after four days	50	100	100
	100	100	50
	200	100	31
	400	20	19

[a] Acclimated activated sludge maintained at pH 7.0 and 26°C.

Source: Adapted from Ingols, Gaffney, and Stevenson, Table III, p. 631. Reprinted by permission of the publisher.

TABLE B.7.8. AMOUNT OF CLAYS REQUIRED TO REDUCE THE CONCENTRATIONS OF 2,4-DICHLOROPHENOL AND 2,4-D COMPOUNDS TO 2.0 mg/liter

Initial concentration (mg/liter)	Compound	Amount of clay (g)		
		Kaolinite	Illite	Bentonite
3	2,4-Dichlorophenol	96.1	19.2	16.4
	2,4-D sodium salt	26.3	35.0	40.0
	2,4-D isopropyl ester	37.6	11.8	6.9
	2,4-D butyl ester	42.7	13.3	8.8
	2,4-D isooctyl ester	44.6	14.2	10.3
4	2,4-Dichlorophenol	192.2	38.5	32.8
	2,4-D sodium salt	52.6	69.9	80.0
	2,4-D isopropyl ester	75.2	23.6	13.8
	2,4-D butyl ester	85.5	26.7	17.5
	2,4-D isooctyl ester	89.2	28.3	20.6
5	2,4-Dichlorophenol	288.3	57.7	49.2
	2,4-D sodium salt	78.9	104.9	120.0
	2,4-D isopropyl ester	113.8	35.4	20.7
	2,4-D butyl ester	128.2	40.0	25.3
	2,4-D isooctyl ester	133.9	42.5	30.9
6	2,4-Dichlorophenol	384.6	76.9	65.6
	2,4-D sodium salt	105.3	129.9	160.0
	2,4-D isopropyl ester	150.4	47.2	27.6
	2,4-D butyl ester	170.9	53.3	35.1
	2,4-D isooctyl ester	178.5	56.6	41.2

Source: Adapted from Aly and Faust, 1964, Table II, p. 543. Reprinted by permission of the publisher.

weak. It is highly probable that the sorption behavior of 2-chlorophenol and 2,4-dichlorophenol is similar to that of 2,4-D. In natural soil systems, sorption may not be extensive, thereby favoring their downward movement in soil with water.

The persistence of 2-chlorophenol in soils was studied by Walker (1954) using the percolation technique. Solutions of 2-chlorophenol (1.0 g) were allowed to percolate through 100 g of a Rothamsted soil (light clay with a pH of 6.8), and the disappearance of the initial and subsequent doses was measured. Two-thirds of the initial dose disappeared in ten days. Disappearance of subsequent doses occurred approximately twice as rapidly as that of the first dose, suggesting microbial participation. Further evidence of microbial decomposition was indicated by the more rapid disappearance of 2-chlorophenol in fresh soil than in sterilized soil within seven days of percolation.

Participation of soil microorganisms in the dissipation of 2-chlorophenol, 2-4-dichlorophenol, and other chlorophenols is also indicated by the investigation of Alexander and Aleem (1961) using suspensions of two silt loam soils. Microbial metabolism of the chemicals was evidenced by more rapid disappearance of incremental additions of the compounds than of initial enrichments. Also, inhibition of degradation occurred with addition of sodium azide, a toxic agent. 2-Chlorophenol and 2,4-dichlorophenol disappeared rapidly in suspensions of Dunkirk and Mardin silt loams; disappearance was faster for the latter soil (Table A.7.2). 2,4-Dichlorophenol was degraded faster than 2,4-D. Sharpee (1973) reported accumulation of 2,4-dichlorophenol during the degradation of 2,4-D in soils, but it did not persist as long as the herbicide.

Although 2-chlorophenol and 2,4-dichlorophenol appear to be short-lived in soils, the data are inconclusive and factors affecting their persistence need further study. However, there are indications that microbial degradation is the major avenue of dissipation for these chemicals in soils. Under field conditions, degradation of 2,4-dichlorophenol, which is more likely to reach the soil system as a contaminant and degradation product of 2,4-D, could be faster than degradation of the herbicide. The role of microorganisms in the degradation of 2,4-D has been conclusively demonstrated (Loos, 1975); under favorable conditions, 2,4-D disappears from soils in about 30 days (Kearney et al., 1969). Warm, moist, well-aerated soils with ample organic matter promote the proliferation of microorganisms known to metabolize 2,4-D.

B.7.3.4 Microbial Decomposition in Soils and Aquatic Environments

As discussed previously, microorganisms may play a major role in the dissipation of chlorophenols in the environment. Therefore, these chemicals could persist longer under conditions unfavorable for microbial growth and activity. In this section, the microorganisms involved and possible degradation pathways for 2-chlorophenol and 2,4-dichlorophenol are discussed.

Limited information indicates that 2-chlorophenol is biodegradable (Walker, 1954; Baird et al., 1974). Several genera of bacteria isolated from soils are capable of metabolizing 2-chlorophenol. *Pseudomonas* sp., *Nocardia* sp., *Mycobacterium coeliacum*, and *Bacillus* sp. can oxidize 2-chlorophenol to 3-chlorocatechol (Spokes and Walker, 1974). The fate of the catechol intermediate was elucidated by a study of the metabolism of 2,4-D by *Pseudomonas* sp. (Evans et al., 1971). 2,4-Dichlorophenoxyacetic acid was used as the sole carbon source for *Pseudomonas* strains isolated from soil. The herbicide was metabolized to 2,4-dichlorophenol, 2-chlorophenol, 3,5-dichlorocatechol, and α-chloromuconate, which was further metabolized to release Cl^- and unidentified metabolites. The appearance in culture of 2-chlorophenol suggests that nonoxidative elimination of chlorine occurred from 2,4-dichlorophenol or possibly from 2,4-D itself. The accumulation of α-chloromuconate is probably a further manifestation of this phenomenon; it is likely formed by the action of the ring cleavage enzyme of 3-chlorocatechol, which, in turn, is derived from either 2-chlorophenol or 3,5-dichlorocatechol. Figure B.7.2 outlines

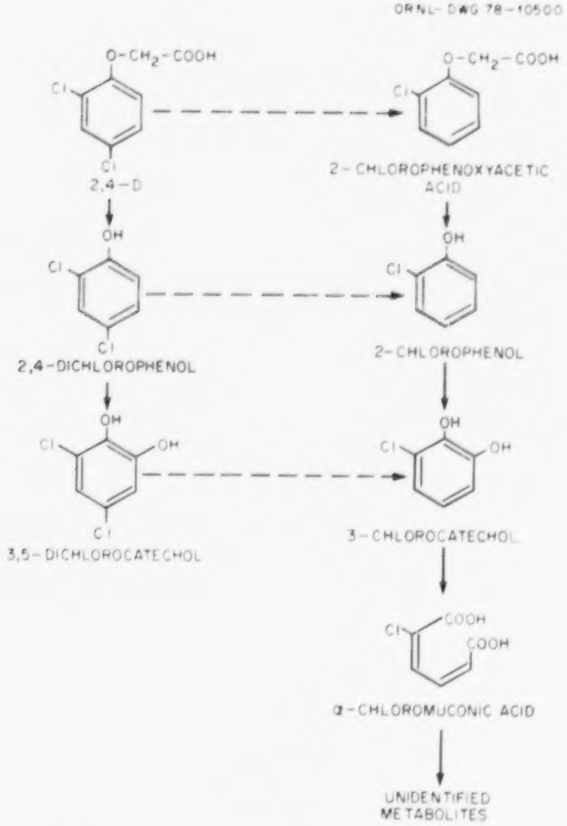
ORNL-DWG 78-10500
O-CH2-COOH
Cl
Cl
2,4-D
O-CH2-COOH
Cl
2-CHLOROPHENOXYACETIC ACID
OH
Cl
Cl
2,4-DICHLOROPHENOL
OH
Cl
2-CHLOROPHENOL
OH
Cl
OH
Cl
3,5-DICHLOROCATECHOL
OH
Cl
OH
3-CHLOROCATECHOL
Cl
COOH
COOH
α-CHLOROMUCONIC ACID
UNIDENTIFIED METABOLITES

Figure B.7.2. Proposed pathway of 2-chlorophenol degradation by *Pseudomonas* sp. Source: Adapted from Evans et al., 1971, Scheme 1, p. 550. Reprinted by permission of the publisher.

a possible pathway of 2-chlorophenol metabolism in microorganisms which involves orthohydroxylation of the ring followed by ring cleavage. Further degradation products have not been identified.

Soil bacteria capable of detoxifying phenoxy herbicides, including *Arthrobacter* sp. and *Pseudomonas* sp., are also active in 2,4-dichlorophenol decomposition. The potential clearly exists for bacterial enzymes adapted to phenoxy herbicides to attack compounds structurally related to the herbicides. Intact cells of *Arthrobacter* sp. are capable of converting 2,4-D and other phenoxyacetates to the corresponding phenols (Loos, Bollag, and Alexander, 1967; Loos, Roberts, and Alexander, 1967*a*, 1967*b*). 2,4-Dichlorophenol formed from 2,4-D is, in turn, oxidized by microbial cells. Bollag et al. (1968) and Bollag, Helling, and Alexander (1968) studied the metabolism of 2,4-dichlorophenol by *Arthrobacter* sp. capable of detoxifying phenoxyacetate. 2,4-Dichlorophenol was converted to 3,5-dichlorocatechol through enzymatic orthohydroxylation (Figure B.7.3). Oxygen and NADPH were required in the reaction. The catechol produced

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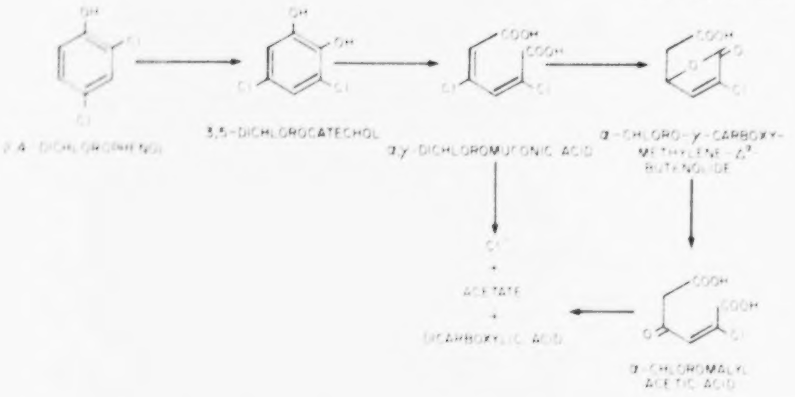

Figure B.7.3. Pathway for degradation of 2,4-dichlorophenol and chlorocatechol by *Arthrobacter* sp. Source: Adapted from Bollag et al., 1968, Figure 5, p. 833. Reprinted by permission of the publisher.

enzymatically from 2,4-dichlorophenol was further metabolized to muconic acid by ortho ring cleavage. Lactonization of muconic acid, involving dehydrochlorination, yielded the butenolide which was delactonized to maleylacetic acids. *Pseudomonas* sp. degraded 2,4-dichlorophenol generated from 2,4-D in a fashion similar to that by *Arthrobacter* sp. (Evans et al., 1971). Other studies showed that the final steps of 2,4-D degradation produce acids (e.g., succinic and presumably acetic acids) that can be metabolized by way of the tricarboxylic acid cycle (Duxbury et al., 1970).

Fungi may also possess the ability to metabolize chlorophenols. Lyr (1962) reported the enzymatic detoxification of chlorophenols, including 2,4-dichlorophenol, by oxidases found in wood-rotting fungi, such as laccase, tyrosinase, and peroxidase. Walker (1973) isolated a phenol-utilizing strain of *Rhodotorula glutinis* from a Rothamsted soil. Phenol-grown cells consumed oxygen in the presence of 2-chlorophenol, 2,4-dichlorophenol, and other chlorophenols. However, this fungus did not grow with chlorophenols as the sole carbon source, which indicates the involvement of a phenol-induced enzyme system. It remains to be determined if fungal strains are capable of utilizing chlorophenols as the sole carbon source and if degradation pathways of fungal metabolism are similar to those of bacterial metabolism.

Aquatic microorganisms have been implicated in the degradation of 2,4-dichlorophenol in activated sludge (Hemmett, 1972) and in aerated pond effluent (Sidwell, 1971). Nachtigall and Butler (1974) isolated and identified *Pseudomonas* sp. and *Nocardia* sp. as the microorganisms responsible for the degradation of chlorophenols in activated sludge. Warburg respirometric studies showed that *Pseudomonas* sp. oxidized all phenols and chlorophenols, including 2-chlorophenol and 2,4-dichlorophenol, but *Nocardia* sp. oxidized only the monochlorophenols, including 2-chlorophenol.

Dioxin formation through microbial condensation of 2-chlorophenol and 2,4-dichlorophenol is of interest because of the high toxicity of dioxins. No evidence of this reaction was observed by Kearney, Woolson, and Ellington (1972) in two soils incubated with 10 to 1000 mg/liter 2,4-dichlorophenol for 70 days. Microbial condensation reactions of lower chlorophenols to form dioxins in the environment appear unlikely. This information is important because biosynthetic production of dioxins from chlorophenols or herbicide metabolites is impossible to regulate under environmental conditions.

Although degradation studies have been performed in vitro, the degradation mechanisms and products of 2-chlorophenol and 2,4-dichlorophenol may be the same in soils and aquatic environments. Apparently, the same microorganisms are responsible for the metabolism of these chemicals. Metabolite accumulation could possibly occur. Sharpee (1973) found that when 2,4-D was added to a freshwater and sediment system, an unknown compound(s) persisted long after the 2,4-D had disappeared. Persistence and accumulation are expected in anaerobic environments.

B.7.3.5 Photodecomposition

Many organic compounds, including chlorophenols, undergo photochemical decomposition when exposed to ultraviolet light. The prime requirement for such reactions is the absorption of ultraviolet light energy by the compounds. Photochemically induced degradation occurs at surfaces of airborne particulates and water. Chlorophenols reach such surfaces by various mechanisms and therefore are subject to exposure to sunlight and are susceptible to photochemical degradation.

The photochemical reaction of 2,4-dichlorophenol is elucidated partially from irradiation studies of 2,4-D. Aly and Faust (1964) demonstrated the rapid decomposition of an aqueous solution of the 2,4-D sodium salt and 2,4-dichlorophenol exposed to ultraviolet light. Losses of 50% were detected within 50 min for 2,4-D and within 5 min for 2,4-dichlorophenol at pH 7.0. Rates of photolysis were lowest at pH 4 and reached a maximum at pH 9. 2,4-Dichlorophenol decomposed more rapidly than 2,4-D at all pH values. Several studies have shown that 2,4-dichlorophenol is the major photolytic product of 2,4-D compounds (Aly and Faust, 1964; Crosby and Tutass, 1966; Boval and Smith, 1973; Zepp et al., 1975) and nitrofen (Nakagawa and Crosby, 1974*a*, 1974*b*). Aly and Faust (1964) noted an increase followed by a decrease in the amounts of 2,4-dichlorophenol produced from 2,4-D compounds with time, suggesting further photolysis of the chemical. Crosby and Tutass (1966) irradiated aqueous solutions of 2,4-D sodium salt with ultraviolet light at 254 nm and identified the photolysis products (Figure B.7.4). The major reaction was the cleavage of the ether bond to produce 2,4-dichlorophenol, which was dehalogenated or hydroxylated under the influence of ultraviolet light to form 4-chlorocatechol and 1,2,4-benzenetriol. The benzenetriol was oxidized and polymerized rapidly to humic acids by a light-independent process. Sunlight irradiation of 2,4-D in water or on filter paper yielded photolytic products qualitatively similar to those formed under ultraviolet irradiation. Photodecomposition of the herbicide nitrofen

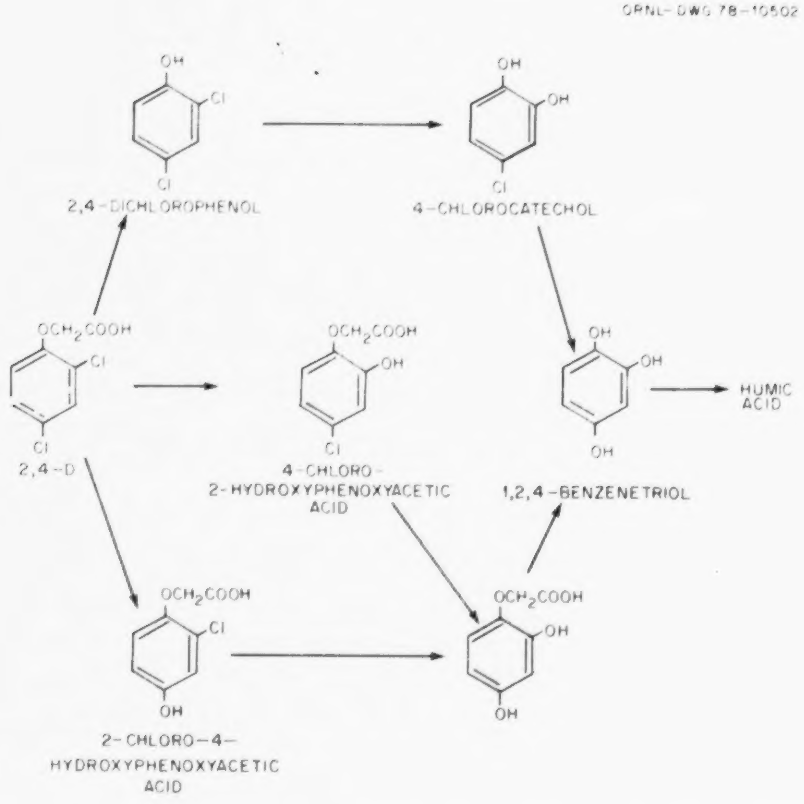
ORNL-DWG 78-10502
OH
Cl
Cl
2,4-DICHLOROPHENOL
OH
OH
Cl
4-CHLOROCATECHOL
OCH2COOH
Cl
Cl
2,4-D
OCH2COOH
OH
Cl
4-CHLORO-2-HYDROXYPHENOXYACETIC ACID
OH
OH
OH
1,2,4-BENZENETRIOL
HUMIC ACID
OCH2COOH
Cl
OH
2-CHLORO-4-HYDROXYPHENOXYACETIC ACID
OCH2COOH
OH
OH

Figure B.7.4. Photolysis pathway for 2,4-D. Source: Adapted from Crosby and Tutass, 1966, Figure 3, p. 599. Reprinted by permission of the publisher.

in aqueous suspensions under sunlight or simulated sunlight (ultraviolet irradiation) also was characterized by rapid cleavage of the ether linkage to give 2,4-dichlorophenol and 4-nitrophenol as the major products (Nakagawa and Crosby, 1974*a*). The 2,4-dichlorophenol arising from nitrofen appeared to follow the same photolytic pathway shown in the investigation of Crosby and Tutass (1966).

The possibility of dioxin formation during photolysis of 2,4-dichlorophenol has been of concern. Plimmer and Klingebiel (1971) studied the products of riboflavin-sensitized photodecomposition of 2,4-dichlorophenol in water irradiated at a wavelength of 280 nm. Major products identified were dimeric compounds; no dioxins were detected. Therefore, it seems unlikely that dioxins will be formed from 2,4-dichlorophenol in aquatic systems.

In natural systems, photochemical reactions are likely to occur simultaneously with adsorption and microbial decomposition. For the chlorophenols, microbial decomposition appears to be the dominant dissipation mechanism. However, photodecomposition possibly contributes to the dissipation of airborne chlorophenols.

B.7.4 WASTE MANAGEMENT

Chlorophenols occur in natural waters and wastewaters through various sources and/or reactions. They are present with other organic compounds in concentrations ranging from low in natural waters to high in wastewaters generated from the manufacture of chlorophenols and phenoxyalkanoic herbicides. In this section, treatment processes applicable to wastewater containing 2-chlorophenol and 2,4-dichlorophenol are discussed along with removal of these chemicals from drinking water.

B.7.4.1 Primary Treatment

Primary treatment consists essentially of settling solids after screening off large materials. Settling may not remove 2-chlorophenol and 2,4-dichlorophenol from water because, as indicated earlier, these chemicals are sorbed poorly on particulate or suspended matter. Chemical coagulation could be employed to improve the settling of solids from wastewater. However, Aly and Faust (1965) reported the ineffectiveness of flocculants such as aluminum sulfate and ferric sulfate in decreasing the concentrations of 2,4-dichlorophenol and 2,4-D in water. Apparently, 2,4-D and 2,4-dichlorophenol stay either in solution or in discrete form, depending on the pH of the wastewater, and are unaffected by coagulants or sorbents. Chlorophenols are dissociated mainly in aqueous solutions with pH values higher than 5 (Cserjesi, 1972).

B.7.4.2 Secondary Treatment

Secondary treatment involves the removal of organic matter from wastewater by biological processes. Biological methods used in waste treatment include trickling filters, activated sludge, and oxidation ponds. These methods all work on the same principle: microorganisms utilize the organic materials in the wastewater as a source of energy and convert them to more simple, less toxic substances. Because 2-chlorophenol and 2,4-dichlorophenol are easily biodegradable, secondary treatment should provide excellent removal of these chemicals.

Mills (1959) removed "dichlorophenol" from a wastewater stream containing 2,4-D by using a pilot plant designed as a combined trickling filter and activated sludge system. The pilot plant unit was seeded with activated sludge from a local sewage plant, and the waste stream was diluted to 10% strength with water before treatment. The average removal of "dichlorophenol" was 86%.

Sidwell (1971) used a combined aerated lagoon and stabilization pond to study the biological treatment of wastes arising from the manufacture of phenoxyalkanoic herbicides, particularly 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The industrial waste, in addition to having a high biochemical oxygen demand and high concentrations of solids and chlorides, contained a mixture of chlorophenols (Tables B.7.2 and B.7.3). During the first four months of this study of the joint treatment of domestic sewage and industrial waste, a period when industrial activity was minimal, the average reduction of chlorophenols across the aeration lagoon and stabilization pond was 92%. During a subsequent period of industrial plant operation, removal of chlorophenols by the aerated lagoon alone ranged from 55% to 89%, and the overall removal of chlorophenols by the lagoon and stabilization pond ranged from 87% to 94%. Removal of chlorophenoxy acids was lower, ranging from 30% to 70% in the lagoon and 44% to 80% in the lagoon and stabilization pond. Most of the chlorophenols are likely to be decomposed in the aeration lagoon. In vitro experiments with individual chlorophenols, including 2,4-dichlorophenol and the related chlorophenoxy acids diluted with aeration effluent, indicated that these compounds are degraded rapidly when a sufficient microbial population has been developed. Obviously, the bacterial population capable of degrading these compounds and the nutrients required by these populations are present in the aeration lagoon effluent.

B.7.4.3 Soil Disposal

As previously discussed (Section B.7.3.4), 2-chlorophenol and 2,4-dichlorophenol are biodegradable by soil organisms. Hence, soil disposal could be employed as a biological method of treating wastes containing chlorophenols. On this basis, Montgomery et al. (1971) investigated the treatment of pesticide wastes resulting from the manufacture of 2,4-D by land application. The waste concentrate consisted primarily of unreacted 2,4-dichlorophenol, unrecovered 2,4-D, and considerable amounts of sodium hydroxide. The waste material was diluted and applied to approximately 3- by 3-m plots at the rate of 900 kg 2,4-dichlorophenol and 300 kg 2,4-D. The plots were watered immediately to insure that the waste material penetrated the soil and was available to microorganisms. Analyses of soil collected at different depths showed significant reduction of 2,4-dichlorophenol and 2,4-D in the first month following application. After this period, the concentrations of 2,4-dichlorophenol and 2,4-D generally declined until about the eleventh month when the last samples were taken. Microbial populations were reduced significantly a short time after application, presumably due to the adverse effect of high concentrations of the chemicals. However, within four to six months, a gradual return to normal populations was observed. Proper maintenance of moisture and temperature accelerated the microbial degradation. Although soil disposal is a promising means of chemical waste treatment, high land values may limit its use.

B.7.4.4 Chemical Oxidation

Chlorination is practiced primarily for disinfecting drinking water and effluents from primary and secondary treatment plants. It also is employed for removal of chlorophenolic tastes and odors caused primarily by monochlorophenols and dichlorophenols. During chlorination of water, 2-chlorophenol could be chlorinated to 2,4-dichlorophenol, which, in turn, is chlorinated to 2,4,6-trichlorophenol by stepwise substitution in the 4 and 6 positions of the aromatic ring (Burttschell et al., 1959; Eisenhauer, 1964; Manufacturing Chemists Association, 1972). The maximum rate of chlorination occurs between pH 7 and 9 (Lee and Morris, 1962).

With excess chlorine, ring oxidation follows the formation of 2,4,6-trichlorophenol and results in the production of oxidized products, including carboxylic acids. The ultimate products do not have offensive tastes or odors.

The use of potassium permanganate for control of tastes and odors has been accepted in water treatment plants. The effective oxidation of 2,4-dichlorophenol by potassium permanganate has been reported. Aly and Faust (1965) found that the amount of 2,4-dichlorophenol oxidized was directly proportional to the amount of the oxidant added. The optimum pH range for the oxidation was 4.0 to 7.0.

B.7.4.5 Ion Exchange

Although sorption of 2,4-dichlorophenol on clay minerals has been shown to be insignificant (Aly and Faust, 1964), 2,4-dichlorophenol can be sorbed to a large extent by ion-exchange resins (Aly and Faust, 1965). Aly and Faust (1965) evaluated the possibility of using ion exchangers to remove 2,4-dichlorophenol from natural waters. In general, the strongly basic anion-exchange resins sorbed more of the chemical than did the cation exchangers. Sorption was not due to the ion-exchange process because no chlorides were released. Perhaps other mechanisms could have been responsible for sorption.

B.7.4.6 Activated Carbon Adsorption

Activated carbon has been used in concentrating low levels of chlorophenols and other organic compounds in water (Eichelberger et al., 1970). Its utility as a water treatment method has been explored. Aly and Faust (1965) effectively sorbed 2,4-dichlorophenol from water employing activated carbon. For an initial concentration of 100 µg/liter, 5.9 µg/liter of carbon was needed to reduce the concentration of 2,4-dichlorophenol to 2 µg/liter.

SECTION B.7

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B.8 ENVIRONMENTAL INTERACTIONS AND THEIR CONSEQUENCES

B.8.1 ENVIRONMENTAL CYCLING OF 2-CHLOROPHENOL AND 2,4-DICHLOROPHENOL

The sources, distribution, and fate of the monochlorophenols and dichlorophenols in soils, aquatic environments, and the atmosphere are discussed in Section B.7. A possible cycling of these chemicals in the environment, indicating the likely sources of contamination, interactions, and sinks, is depicted in Figure A.8.1. Caution should be used in the interpretation of this cycle because only scanty data are available, which makes it difficult to establish any pattern of distribution and flow of the chemicals into various components of the environment.

Soil and aquatic systems could be contaminated through direct usage of the chemicals or closely related compounds, discharge of industrial wastes and sewage, and chlorination of water containing phenols. Although no documentation is available on the relative contribution from each source, contamination likely occurs mainly from application of 2,4-dichlorophenoxyacetic acid (2,4-D), which contains 2,4-dichlorophenol as a contaminant or degradation product or from discharge of chlorophenolic wastes generated from chemical plants manufacturing phenoxyalkanoic herbicides. The monochlorophenols and dichlorophenols are quite volatile and, when introduced into the environment, are susceptible to volatilization. Data on their presence in the atmosphere and their subsequent redeposition during precipitation and fallout are nonexistent.

Like many other organic compounds, 2-chlorophenol and 2,4-dichlorophenol undergo physicochemical and biological interactions with various components of the environment. These chemicals can be sorbed by soils, sediments, and airborne particulate materials. Sorption plays a major role in the downward and overland movement of contaminants from soil surfaces. Those compounds that are tightly bound tend to associate with the eroded soil during runoff, and weakly bound compounds may move with the water phase during infiltration. Limited evidence indicates that sorption of 2,4-dichlorophenol by clay minerals is insignificant (Aly and Faust, 1965) so that the compound could move through the soil profile. Thus, minimal losses are likely to be associated with erosion from agricultural lands. The rates of chemical and biological transformations of chlorophenols determine their persistence and subsequent degree of movement in a particular segment of the environment. Chemical oxidation occurs during treatment of wastewater (Section B.7.4.4); photodecomposition is possible when the chemicals are present on surfaces of water, soil, and airborne particulates exposed to sunlight (Section B.7.3.5). Although data are limited and fragmentary, 2-chlorophenol and 2,4-dichlorophenol are assimilated by soil and aquatic microorganisms (Section B.7.3), absorbed by plants (Section B.4), and absorbed and/or ingested by mammals (Sections B.5 and B.6). Microbial degradation appears to be the principal mechanism of removal and the principal sink. The degree of metabolism and mode of distribution of absorbed or ingested monochlorophenols and dichlorophenols in plants, wildlife, and mammals can influence bioaccumulation. Bioaccumulated chlorophenols, if they persist, can be returned either to the soil and water systems during decay and excretion or can be consumed by higher organisms, including humans, through the food chain.

B.8.2 2-CHLOROPHENOL AND 2,4-DICHLOROPHENOL

Available data indicate that contamination of food with 2-chlorophenol and 2,4-dichlorophenol is minimal; thus, transfer to humans through the food chain appears to be remote. Although 2-chlorophenol and 2,4-dichlorophenol are not used directly in agricultural production, they may find their way into crops through an indirect means — the metabolism of plant-assimilated 2,4-D. A few reports indicated that 2,4-dichlorophenol was present in potato tubers and rice plants treated with 2,4-D (Bristol et al., 1974; Sokolov et al., 1974). In stored potato tubers, low levels of 2,4-dichlorophenol were detected (Bristol et al., 1974). Large quantities of 2,4-dichlorophenol were found in rice plants; however, rice grains contained no residue of the compound at maturity (Sokolov et al., 1974). Isensee and Jones (1971) demonstrated the absorption of 2,4-dichlorophenol by soybeans and oats from soil. Negligible amounts of 2,4-dichlorophenol were detected in oat grains and soybean seeds at maturity, indicating that the chemical was not accumulated.

Domestic animals, including poultry, may ingest feeds containing pesticides or drink water contaminated directly with 2-chlorophenol and 2,4-dichlorophenol. Some studies showed the appearance and distribution of 2,4-dichlorophenol in tissues of animals fed with 2,4-D and nemacide. Muscle and fat tissues of sheep and cattle which consumed feed containing high concentrations of 2,4-D (300 to 2000 mg/kg) showed no detectable 2,4-dichlorophenol, but parts-per-million levels of the metabolite were evident in the liver and kidney (Clark et al., 1975). Sherman, Beck, and Herrick (1972) found no detectable residues of 2,4-dichlorophenol in fat or in breast muscle of laying hens administered feeds containing 100 to 800 mg/kg nemacide. The liver and egg yolk contained appreciable quantities of 2,4-dichlorophenol. These limited data indicate that although 2,4-dichlorophenol tends to accumulate in liver and kidney, it is not found in meats even at extremely high dosages of the pesticides. Furthermore, Bjerke et al. (1972) reported no contamination of milk and cream from cows dosed with feeds containing 100 to 1000 mg/kg 2,4-D.

Apparently direct exposure of humans to these chemicals during manufacture constitutes the major hazard. Widespread distribution and accumulation of 2-chlorophenol and 2,4-dichlorophenol in the environment is unlikely because these compounds tend to degrade quite easily in soil and aquatic systems. However, data are not conclusive; more definitive investigations are needed to assess judiciously the environmental hazards associated with the use of 2-chlorophenol and 2,4-dichlorophenol. For example, accumulation in fish and other aquatic organisms exposed to waters contaminated with the chemicals as a result of discharge of chlorinated effluents from sewage and industrial waste treatments has not been investigated.

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PART C

2,4,5-TRICHLOROPHENOL, 2,4,6-TRICHLOROPHENOL, AND TETRACHLOROPHENOLS

C.1 SUMMARY

C.1.1 DISCUSSION OF FINDINGS

Only three trichlorophenol and tetrachlorophenol isomers have commercial importance. 2,4,6-Trichlorophenol is used as a bactericide and fungicide for preserving wood, leather, and glue and for treating mildew on textiles. 2,4,5-Trichlorophenol and its sodium salts have been used directly as germicides and as primary compounds in the synthesis of a number of pesticides, including the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its derivatives. Most of the 2,4,5-trichlorophenol produced in the United States is used as an intermediate for the synthesis of these pesticides. 2,3,4,6-Tetrachlorophenol, the only tetrachlorophenol isomer of commercial importance, is used as a germicide for the preservation of wood, latex, and leather and as an insecticide.

The three compounds have many similar physical and chemical properties. Each is a solid at room temperature and has very low solubility in water but is readily soluble in a number of organic solvents. Each compound is volatile with steam and to some extent from aqueous solution. Chemical reactions of these compounds which may occur in the environment are largely unknown.

A number of analytical techniques have been used to identify and quantify trichlorophenols and tetrachlorophenols in soil, water, and biological tissue samples. Gas-liquid chromatography, employing either electron-capture or flame-ionization detectors, is the most widely used technique for separating, identifying, and quantifying chlorophenols. Because gas-liquid chromatography is sensitive to nanogram levels of these compounds, it is adaptable for the quantification of trace amounts. Colorimetric methods, although simple and rapid, lack specificity and thus are only useful for semiquantitative determinations. Other less sensitive methods of detection — including thin-layer chromatography, infrared and ultraviolet spectrophotometry, and mass spectrometry — are useful only when large concentrations of the compounds are present.

The mechanism of toxicity of higher chlorophenols to microorganisms is not completely understood. It is believed that they affect the cell membrane, with subsequent leakage of cell constituents, and perhaps inactivate specific enzyme systems. Because they have low water solubility and high lipid solubility, their gross effect on bacteria and fungi is likely due to association with cellular lipids and subsequent alteration of cell permeability. Exposure of bacteria to 2,4-6-trichlorophenol causes leakage of certain cell constituents through the cell membrane; 2,4,6-trichlorophenol also inhibits the enzyme catalase. In addition, higher chlorophenols, particularly tetrachlorophenols, can inhibit oxidative phosphorylation. The contributions of these mechanisms to the toxicity of higher chlorophenols to microorganisms is unknown.

All chlorophenols possess some bactericidal properties which generally increase with the degree of chlorine substitution up to the trichlorophenol derivatives. The tetrachlorophenol isomers are considerably less toxic to bacteria than the trichlorophenol isomers; however, tetrachlorophenols possess enhanced fungicidal activity. The pH of the media has a profound effect on the bactericidal efficiency. For example, 2,4,6-trichlorophenol is most effective in inhibiting growth of bacteria in acidic systems, indicating that the undissociated compound is more germicidal than the dissociated form.

Many fungi are susceptible to the toxic effects of 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol. A pH-dependent effect on toxicity, similar to that with bacteria, exists. The unionized form is apparently more toxic to fungi than the ionized form. The pH effect is important not only in assessing the degree of toxicity of these compounds to various fungi, but also in determining the effectiveness of the compounds as germicides.

Both 2,4,5- and 2,4,6-trichlorophenol are toxic to algae. The mechanism of this toxicity is not known, but both compounds pose a serious hazard to algae in waste stabilization ponds. Care must be taken that they are not dumped or allowed to seep into such waste treatment facilities. No information on the algicidal properties of tetrachlorophenols is available. However, it is expected that they pose a similar threat to healthy algae growth.

Very little information on biological aspects of trichlorophenols and tetrachlorophenols in vascular plants is available. 2,4,5-Trichlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol are toxic to the aquatic plant *Lemna minor*. 2,3,4,6-Tetrachlorophenol is three times more toxic than 2,4,5-trichlorophenol, which is, in turn, approximately twice as toxic as 2,4,6-trichlorophenol. 2,3,4,6-Tetrachlorophenol and tetrachlorophenol conjugates have been detected in lettuce plants grown in nutrient solutions containing lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane). The nature of the conjugates and their role in the biodegradation of 2,3,4,6-tetrachlorophenol are unknown.

Foliar application of 2,3,5-trichlorophenol increases the percentage of mitotic abnormalities in *Vicia faba* plants. No effect on yield has been noted, and the biological significance of the increased mitotic abnormality rate is not known. 2,4,6-Trichlorophenol and tetrachlorophenols were not tested in this system.

No documented toxic effects of trichlorophenols and tetrachlorophenols on domestic animals and wildlife were found. No effect was noted in cattle fed 2,4,5-trichlorophenol at levels up to 159 mg/kg body weight for 78 days. The tetrachlorophenol isomers which can uncouple oxidative phosphorylation may pose a hazard to domestic animals and wildlife if the compound reaches the environment in large amounts. Toxicosis is speculated to resemble the clinical picture of pentachlorophenol poisoning, but no data on potential effects are available.

The higher chlorophenols may appear in animal tissues following absorption of other organic compounds. Trichlorophenols and tetrachlorophenols have been detected in animal tissues following administration of 2,4,5-T, silvex, lindane, ronnel, and erbon. The potential for toxic effects due to the presence of the pesticide metabolites are not known. No toxic effects from the presence of chlorophenols produced from pesticides in the animal have been found. Hazards to humans as consumers of milk and meat have not been assessed. Demonstrable amounts of 2,4,5-trichlorophenol were detected in the milk and cream of cows fed a diet containing 300 to 1000 mg/kg of the herbicide 2,4,5-T.

The presence of musty taint in chickens and chicken eggs has been ascribed to absorption of chloroanisoles by chickens from litter contaminated with chlorophenols. Fungi in the litter readily methylate 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol to trichloroanisoles and tetrachloroanisoles which are subsequently absorbed by chickens and produce musty taint. Thus, the presence of these chlorophenols in chicken houses should be scrupulously avoided. Consumption of tainted chickens by humans is unlikely because of the lack of palatability.

Of the trichlorophenol and tetrachlorophenol isomers, only 2,4,6-trichlorophenol has documented toxicity for aquatic organisms. Laboratory experiments indicate that 2,4,6-trichlorophenol is toxic to fish and aquatic microfauna. Oxygen consumption and cell division in fertilized sea urchin eggs are affected by concentrations of 2,4,6-trichlorophenol greater than 6.2 mg/liter. The tetrachlorophenol isomers are probably as toxic to aquatic organisms as pentachlorophenol (Section D.5.2.2). Pentachlorophenol is known to accumulate in aquatic organisms, and tetrachlorophenols, in view of their similar lipid solubility and other chemical and physical characteristics, may share this property.

Cutaneous contact with any chlorophenols should be avoided. The trichlorophenol and tetrachlorophenol isomers are primary skin irritants. Although there is no apparent danger from cutaneous absorption of 2,4,5- or 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol dissolved in organic solvents may be absorbed directly through the skin in acutely toxic amounts. Residues of 2,4,5- and 2,4,6-trichlorophenol and several tetrachlorophenol isomers have been detected in the muscle, liver, and kidney of experimental animals dosed with chemically similar organic compounds (e.g., lindane and 2,4,5-T). The metabolic breakdown of higher chlorophenols in humans is speculative. 2,4,5-Trichlorophenol, 2,4,6-trichlorophenol, and tetrachlorophenols are excreted primarily in the urine, and conjugation to sulfuric and glucuronic acids may occur. Following a one-week exposure to lindane, 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,5- and 2,3,4,6-tetrachlorophenol were excreted in the urine for at least one month; other studies indicated more rapid excretion.

Although documented cases of human toxicity due to trichlorophenol or tetrachlorophenol exposure have not been reported, investigations with experimental animals indicate that 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6- and 2,3,5,6-tetrachlorophenol are lethal when administered orally or parenterally in large amounts. Although mechanisms of toxicity are unclear, trichlorophenols and tetrachlorophenols possess the ability to uncouple oxidative phosphorylation in higher animals; clinical symptoms resemble those seen with acute pentachlorophenol poisoning. Additionally, 2,4,6-trichlorophenol has a convulsive action (similar to phenol) in experimental animals. A general property of uncouplers is the ability to inhibit other enzyme systems in the cell, an effect likely to cause acute poisoning and death in experimental animals. 2,4,5-Trichlorophenol and 2,4,6-trichlorophenol appear to be the least toxic of the higher chlorophenols. In experimental animals, LD_{50} values as high as 3 g/kg body weight have been reported, and chronic feeding of 2,4,5-trichlorophenol at levels of 1 g/kg results only in minor liver and kidney damage in rats. The trichlorophenols are considered generally to be mildly toxic; tetrachlorophenols are considered to be more toxic, with an LD_{50} in experimental animals ranging from 120 to 250 mg/kg. Tetrachlorophenols are moderately toxic when ingested.

Long-term exposure to trichlorophenol formulations has caused chloracne. The highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin may be present as an impurity in technical grades of 2,4,5-trichlorophenol and is capable of causing marked chloracne; therefore, this impurity may be responsible for chloracne development among industrial workers exposed to trichlorophenol formulations. Employees involved in the manufacture of these compounds are exposed to a variety of compounds, and the symptoms cannot be clearly assigned to a specific chlorophenol.

2,4,5-Trichlorophenol promotes the appearance of benign and malignant skin tumors in mice following an initiating dose of dimethylbenzanthracene, but it does not appear to be tumorigenic in the absence of an initiator. No tumor-promoting activity was seen when 2,4,6-trichlorophenol alone was tested. No information on the possible tumorigenic effects of tetrachlorophenols was found.

No effect was seen when 2,4,5-trichlorophenol was tested for embryo toxicity or tetratogenicity in mice at daily dose rates as high as 9 mg/kg body weight. Information on teratogenic effects of 2,4,6-trichlorophenol or tetrachlorophenols was not found.

Although monitoring data on the presence of trichlorophenols and tetrachlorophenols in air, water, or soil are not available, the relatively high volatility of these compounds suggests that volatilization is a major dispersal mechanism of the chemicals into the atmosphere. The potential for large discharges exists in industrial plants where these chlorophenols and the phenoxyalkanoic herbicides are manufactured. Incineration could also generate volatile products through the burning of containers or trash containing chlorophenols. Movement, persistence, and fate of the compounds in the atmosphere are not understood; photodecomposition possibly contributes to their dissipation.

Contamination of aquatic environments with trichlorophenols and tetrachlorophenols may arise from chlorination of phenol or lower chlorophenols in natural water or in primary or secondary effluents of waste treatment plants or from direct addition of the chemicals from industrial sites to waterways [e.g., waste from the manufacture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T showed considerable amounts of 2,4,5- and 2,4,6-trichlorophenol]. Other possible sources are chemical spills, washing of containers and drums in which chlorophenols and 2,4,5-T (or similar herbicides) are stored, degradation products of silvex used for aquatic weed control, and wet and dry atmospheric fallout. Additionally, runoff from urban and agricultural watersheds could be an important nonpoint source of chlorophenols in aquatic environments. Primary sources include degradation products of 2,4,5-T, silvex, and related herbicides which are widely applied in the environment. The exact contribution of runoff to chlorophenol levels in water is not known.

Chlorophenols may be transported in aquatic environments in the dissolved form, associated with suspended matter or bottom sediments, or absorbed in biological tissues. Limited evidence indicates the rapid microbiological degradation of 2,4,5- and 2,4,6-trichlorophenol in aquatic environments and in activated sludge and aeration lagoon effluent. Thus, trichlorophenols do not appear to pose a serious hazard in waste treatment facilities normally exposed to them. Certain bacterial species capable of metabolizing 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol were derived from an activated sludge after cultures were acclimated to the presence of chlorophenol. It is not known if species of bacteria capable of metabolizing higher chlorophenols are widespread in nature. The danger that these compounds may interfere with sewage treatment processes, particularly under shock-loading conditions, cannot be discounted. For example, oxygen uptake in mixed microbial populations from a sewage treatment plant was inhibited at 2,4,6-trichlorophenol concentrations of 50 and 100 mg/liter.

Factors such as oxygen depletion, pH, temperature, concentration of the compound, and composition of the bacterial population affect the degradation rates of trichlorophenols and tetrachlorophenols in aquatic systems. No data are available on microbial decomposition of tetrachlorophenols in aquatic environments. Photodecomposition, which may contribute to the dissipation of chlorophenols from aquatic systems, has been demonstrated for closely related compounds such as 2,4,5-T. Although microorganisms probably play the crucial role in degradation, solar radiation may contribute to degradation in water of sufficient clarity to allow penetration of ultraviolet radiation.

Direct contamination of soils by trichlorophenols and tetrachlorophenols is probably minimal. For 2,4,5-trichlorophenol, the primary source of contamination is from application of 2,4,5-T and silvex. Although data are not available, atmospheric fallout or washout could also contribute to soil contamination.

Preliminary data indicate that trichlorophenols and tetrachlorophenols are probably not tightly bound to soil particles. Under appropriate conditions, leaching through the soil profile is expected to occur, but groundwater contamination is not documented. Persistence and degradation of trichlorophenol and tetrachlorophenol isomers in soil have not been well studied. The participation of soil microorganisms in decomposition has been demonstrated, but it is not known if bacteria capable of metabolizing higher chlorophenols are widely distributed in the environment. Chlorophenol isomers with a chlorine atom in the meta position show marked persistence. 2,4,6-Trichlorophenol is removed rapidly from soil, but 2,4,5-trichlorophenol and 2,3,4,5-tetrachlorophenol can persist for more than 72 days. The reason for higher resistance to degradation by chlorophenols containing chlorine in the meta position is not known.

Of the trichlorophenol and tetrachlorophenol isomers likely to contaminate soil, 2,4,5-trichlorophenol poses the greatest hazard with respect to accumulation and persistence. Persistence of any chemical of course depends on the environmental conditions which affect microbial growth and activity.

A number of fungal species isolated from poultry litter readily degrade 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol to form the corresponding anisoles. No information on possible degradation of 2,4,5-trichlorophenol by fungi is available. The contribution of fungi to the dissipation of trichlorophenols and tetrachlorophenols in the environment has not been determined.

Chlorination of phenol, monochlorophenols, and dichlorophenols reportedly results in stepwise chlorination of the molecule up to the trichlorophenol isomers. Further chlorination apparently results in ring cleavage and the formation of a mixture of nonphenolic products. Ring cleavage probably depends on optimum conditions for chlorination. Pentachlorophenol may be synthesized when phenol is chlorinated under appropriate conditions. Thus, in many cases chlorination of wastewater or drinking water may result in the formation of chlorophenols rather than in the destruction of these potentially hazardous compounds.

Food contamination by trichlorophenols and tetrachlorophenols may occur if residues accumulate and persist in edible portions of crops, fish, or domestic animals. Domestic animals and agricultural crops could also become contaminated indirectly with trichlorophenols and tetrachlorophenols by absorption and/or ingestion followed by degradation of the pesticides 2,4,5-T, silvex, lindane, and pentachlorophenol. For example, lindane absorbed by lettuce plants is metabolized to free and conjugated 2,3,4,6-tetrachlorophenol. No information is available, however, on the presence and metabolic fate of trichlorophenols and tetrachlorophenols in field-grown crops arising from the absorption and eventual metabolism of the above pesticides. Residues of 2,4,5-trichlorophenol have been found in the kidney and liver of sheep and cattle fed very high concentrations of 2,4,5-T and silvex. 2,4,5-Trichlorophenol has also been found in milk and cream of cows fed diets containing large amounts (1000 mg/kg body weight) of 2,4,5-T. It seems unlikely, however, that the large quantities of 2,4,5-T and silvex required to produce detectable concentrations of 2,4,5-trichlorophenol in domestic animals are likely to be found in feed, forage, and pastures. Proper usage of the herbicide should result in negligible residues of 2,4,5-trichlorophenol in meat and milk. Direct exposure of domestic animals to trichlorophenols and tetrachlorophenols seems unlikely; however, some low-level contamination may result when herbicide formulations contaminated with chlorophenols are applied to fields and pastures. The possibility that trichlorophenols or tetrachlorophenols may accumulate in fish and other edible aquatic organisms has not been well studied. If this phenomenon does occur, tetrachlorophenols would be the most likely candidates for accumulation in aquatic organisms. This question warrants further study.

Because musty taint in chickens may result from exposure to chlorophenols, wood shavings which contain chlorophenols should not be used as litter material for broiler chickens or domestic animals. Unfortunately, wood shavings are used extensively as a cheap source of litter for broiler chickens, turkeys, ducks, pigs, and cattle; thus, the danger that preservative-treated wood may be included in these shavings exists. Furthermore, spent litter is being used increasingly as a constituent of animal feeds. Despite this potential hazard, limited information indicates that contamination of foodstuff by residues of trichlorophenols and tetrachlorophenols is unlikely, and transfer to humans through the food chain appears to be remote. Direct exposure during the manufacture of these compounds apparently constitutes the major hazard.

No information is available on the long-term chronic toxicity of 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol; therefore, the degree of hazard posed by chronic exposure is speculative. 2,3,4,6-Tetrachlorophenol is the most toxic of these compounds, but it apparently degrades readily in soil and aquatic environments. No data are available, however, to conclusively prove these contentions. More definitive investigations are needed to assess the environmental hazards associated with use of these compounds.

Another possible hazard associated with the use of chlorophenols is that these compounds may be contaminated with, or give rise to, chlorodibenzo-*p*-dioxins. Some chlorodioxins are known to have extremely high toxicity and teratogenicity, and their presence, even in extremely low quantities, poses a definite hazard. Chlorodioxins associated with the use of trichlorophenols and tetrachlorophenols could be introduced into the environment in two ways: (1) by contamination of commercially produced higher chlorophenols with chlorodioxins and (2) by pyrolytic condensation of higher chlorophenols to chlorodioxins following incomplete incineration of solid waste containing chlorophenols, especially wood. Modern procedures reduce the formation of chlorodioxins during chlorophenol manufacture, but possible production of chlorodioxins during pyrolysis or through presently unknown reaction pathways has not been explored and warrants further consideration.

C.1.2 CONCLUSIONS

1. Gas-liquid chromatography is the best method for the separation, identification, and quantification of low levels of 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and tetrachlorophenols in environmental samples.

2. 2,4,5-Trichlorophenol, 2,4,6-trichlorophenol, and tetrachlorophenols are toxic to microorganisms; effects on cell permeability and oxidative phosphorylation are probable mechanisms of action.

3. Microbial toxicity is pH dependent; the undissociated form of chlorophenol is more toxic than the dissociated form.

4. Limited information indicates that highly chlorinated phenols are toxic to vascular plants. Toxicity roughly corresponds with the degree of chlorination: 2,3,4,6-tetrachlorophenol > 2,4,5-trichlorophenol > 2,4,6-trichlorophenol.

5. No documented toxic effects of trichlorophenols and tetrachlorophenols on domestic animals or wildlife were found.

6. Trichlorophenols and tetrachlorophenols may appear in domestic animals following absorption of chemically related herbicides (e.g., 2,4,5-T, silvex, lindane, ronnel, and erbon).

7. Musty taint may occur in broiler chickens when trichlorophenols or tetrachlorophenols in chicken litter are methylated (with production of the corresponding anisoles) by fungi and absorbed by the chickens.

8. Information on the toxicity of trichlorophenols and tetrachlorophenols to aquatic organisms is needed.

9. Documented cases of human systemic toxicity due to exposure to trichlorophenols or tetrachlorophenols are not available.

10. 2,4,5-Trichlorophenol and 2,4,6-trichlorophenol are not readily absorbed through the skin, but 2,3,4,6-tetrachlorophenol dissolved in organic solvents may be absorbed directly through the skin in acutely toxic amounts.

11. Trichlorophenols are mildly toxic (LD_{50} values as high as 3 g/kg body weight in rats), but tetrachlorophenols pose a more severe acute toxicity hazard (LD_{50} values between 120 and 250 mg/kg body weight in experimental mammals).

12. Long-term exposure to trichlorophenol formulations has caused chloracne in humans, but affected individuals were exposed to a wide variety of chemicals (including 2,3,7,8-tetrachlorodibenzo-p-dioxin, a known impurity in technical chlorophenol formulations, which can cause chloracne).

13. 2,4,5-Trichlorophenol promotes the appearance of tumors in mice following an initiating dose of dimethylbenzanthracene but does not cause tumors in the absence of an initiator. No tumor-promoting activity was found for 2,4,6-trichlorophenol tested in the same system.

14. 2,4,5-Trichlorophenol is not teratogenic or embryo toxic to rats. 2,4,6-Trichlorophenol and tetrachlorophenols have not been tested for these effects.

15. Monitoring data on the presence of trichlorophenols and tetrachlorophenols in air, water, or soil are not available.

16. Likely sources of trichlorophenol and tetrachlorophenol contamination of soil and water are (1) chlorination of phenol or the lower chlorophenols during wastewater or drinking water chlorination procedures, (2) direct addition of the chemicals from industrial sites to waterways, and (3) degradation products of commonly used pesticides (e.g., 2,4,5-T and silvex).

17. Limited evidence indicates the rapid microbiological degradation of 2,4,5- and 2,4,6-trichlorophenol in aquatic environments, activated sludge, and aeration lagoon effluent.

18. More research is warranted on the persistence and degradation of tetrachlorophenols in soils and water.

19. Disruption of normal sewage treatment processes may occur if trichlorophenols or tetrachlorophenols are dumped or allowed to seep into waste treatment facilities not normally exposed to the compounds.

20. Preliminary data indicate that trichlorophenols and tetrachlorophenols are probably not tightly bound to soil particles, and under appropriate conditions, leaching through the soil profile is expected. Groundwater contamination has not been documented.

21. Chlorine substitution in the meta position appears to interfere with microbial decomposition of chlorophenols; thus, 2,4,5-trichlorophenol will probably persist much longer in the environment than either 2,4,6-trichlorophenol or 2,3,4,6-tetrachlorophenol.

22. Chronic toxicity of trichlorophenols or tetrachlorophenols to humans or other organisms has not been documented.

23. Food contamination with trichlorophenols and tetrachlorophenols is minimal, and transfer to humans through the food chain appears to be remote.

24. The environmental hazards posed by commonly found impurities in chlorophenol formulations (i.e., chlorodibenzo-*p*-dioxins) need further assessment.

C.2 CHEMICAL AND PHYSICAL PROPERTIES AND ANALYSIS

C.2.1 PHYSICAL PROPERTIES

Some physical properties of trichlorophenols and tetrachlorophenols are given in Table A.2.1. These chemicals are insoluble in water but readily dissolve in many organic solvents (Doedens, 1963). This property provides a method of isolating the compounds for analysis. All trichlorophenols and tetrachlorophenols behave as weak acids, although the latter are stronger acids. Furthermore, tetrachlorophenols may volatilize less than trichlorophenols.

C.2.2 MANUFACTURE

The commercial synthesis of 2,4,6-trichlorophenol is readily accomplished by the direct chlorination of phenol (Doedens, 1963). Monochlorophenols and dichlorophenols obtained as by-products are removed either by fractional distillation or are converted to the desired product by recycling and further chlorination. Manufacturers of 4-chlorophenol and 2,4-dichlorophenol dispose of the by-products 2-chlorophenol and 2,6-dichlorophenol by chlorinating them to 2,4,6-trichlorophenol. 2,4,6-Trichlorophenol has also been prepared by the diazotization of 2,4,6-trichloroaniline followed by the hydrolysis of diazonium salt. The production of 2,4,5-trichlorophenol involves the hydrolysis of 1,2,4,5-tetrachlorobenzene in the presence of methanol and sodium hydroxide (Doedens, 1963). After hydrolysis the main impurity is 2,4,5-trichloroanisole, which is formed by the reaction of 2,4,5-trichlorophenol and methanol; it is removed by organic solvent extraction and 2,4,5-trichlorophenol is recovered by distillation. This isomer may also be prepared by the diazotization of 2,4,5-trichloroaniline.

2,3,4,6-Tetrachlorophenol can be prepared by direct chlorination of phenol with catalysts such as iodine and iron chloride (Doedens, 1963). However, in industry, the starting materials for its production may be a mixture of 2-chlorophenol, 2,6-dichlorophenol, and 2,4,6-trichlorophenol, which are available if the company is a primary producer of 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol. The compound can also be synthesized by reducing 1,2,4,4,5,6,6-heptachlorocyclohexen-1-one-3 with stannous chloride/hydrochloric acid/acetic acid or with potassium iodide/acetic acid. The preparation of 2,3,4,5-tetrachlorophenol is accomplished either by diazotization of 2,3,4,5-tetrachloroaniline or by hydrolysis of pentachlorobenzene in the presence of methanol and sodium methylate (Doedens, 1963). 2,3,5,6-Tetrachlorophenol can be synthesized from 2,3,5,6-tetrachloroaniline through diazotization or as a coproduct with 2,3,4,5-tetrachlorophenol in the hydrolysis of pentachlorobenzene (Doedens, 1963).

C.2.3 USES

2,4,6-Trichlorophenol is used as a bactercide and fungicide in the preservation of wood, leather, and glue and in the treatment of mildew on textiles (Doedens, 1963; Dow Chemical Company, undated). Its effectiveness is reported to be higher than that of monochlorophenols and dichlorophenols. It is also useful as an ingredient in the preparation of insecticides and soap germicides. The primary use of 2,4,5-trichlorophenol is as an intermediate for the synthesis of pesticides, notably the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its derivatives (Doedens, 1963; Dow Chemical Company, undated). This herbicide provides effective brush control, and it was used widely in Vietnam for defoliation. 2,4,5-Trichlorophenol and its sodium salts are also used directly as germicides and as ingredients of soap germicides.

The chief applications of 2,3,4,6-tetrachlorophenol and its salts include use as germicides for the preservation of wood, latex, and leather and as insecticides (Doedens, 1963). 2,3,4,5-Tetrachlorophenol and 2,3,5,6-tetrachlorophenol do not have any commercial importance (Doedens, 1963).

C.2.4 CHEMICAL REACTIVITY

Electrophilic substitution readily occurs in the aromatic ring of 2,4,6-trichlorophenol through halogenation (Doedens, 1963). Chlorination results in the formation of 2,3,4,6-tetrachlorophenol and pentachlorophenol; bromination yields the monosubstituted and disubstituted derivatives. Direct nitration is difficult because nitration reagents easily oxidize the compound to the benzoquinone derivative. Treatment of 2,4,6-trichlorophenol with chlorine and fuming sulfuric acid results in preparation of the insecticide chloranil (2,3,5,6-tetrachloro-1,4-benzoquinone). 2,4,6-Trichlorophenol reacts with formaldehyde to form a bis (methylene) derivative and with sulfuric chloride to give 3,3'-dihydroxy-2,4,6,2',4',6'-hexachlorodiphenyl sulfide, both of which are soap germicides. The phenolic group of 2,4,6-trichlorophenol reacts like phenol; ethers, esters, amine compounds, and metallic salts have been reported (Doedens, 1963).

Substitution can also occur in the aromatic ring of 2,4,5-trichlorophenol. It can be brominated and nitrated to the monosubstituted and disubstituted derivatives (Doedens, 1963). 2,4,5-Trichlorophenol reacts with formaldehyde and sulfuryl chloride to yield the bis (methylene) and thiobis derivatives, respectively, both of which are soap germicides. The phenolic group of 2,4,5-trichlorophenol undergoes interesting reactions which yield important pesticidal compounds. It reacts with thiophosphoryl chloride and then with sodium methylate in the presence of methanol to produce ronnel [*O*,*O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate], a phosphate insecticide. The manufacture of 2,4,5-T involves etherification of 2,4,5-trichlorophenol with chloroacetic acid in the presence of sodium hydroxide. Reaction of 2,4,5-trichlorophenol with 2-monochloropropionic acid yields silvex [2-(2,4,5-trichlorophenoxy)-propionic acid]. Metal trichlorophenates are prepared by reacting 2,4,5-trichlorophenol with metal salts in the presence of organic solvents (Doedens, 1963).

Salts of 2,3,4,6-tetrachlorophenol are manufactured easily by reacting the compound with metal salts in mixtures of organic solvents (Doedens, 1963).

In summary, trichlorophenols undergo several reactions which yield important products such as pesticides and soap germicides. Salts of 2,4,6- and 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol are readily prepared. In salt form the chemicals are more soluble in water. Apparently, the reactions described do not occur in the natural environment. One reaction that 2,4,6-trichlorophenol may undergo in water is oxidation to hydroquinone during chlorination (Burttschell et al., 1959).

C.2.5 TRANSPORT AND TRANSFORMATION IN THE ENVIRONMENT

The transport and transformation mechanisms of 2,4,5- and 2,4,6-trichlorophenol (use of the term trichlorophenol in the subsequent discussion refers only to these isomers) and of tetrachlorophenols in air, soil, and aquatic environments are discussed fully in Section C.7. A summary is presented here to give perspective on the possible relationship of the physical and chemical properties of the chemicals to their interactions with the different segments of the environment.

C.2.5.1 Air

Trichlorophenols and tetrachlorophenols are relatively volatile, and volatilization is probably the major dispersal mechanism of the chemicals to the atmosphere. However, monitoring data are lacking, making it difficult to determine transportation and transformation mechanisms.

C.2.5.2 Aquatic Environment

Trichlorophenols and tetrachlorophenols may be present in the aquatic environment either in dissolved form, associated with suspended matter or bottom sediments, or absorbed by organisms. Metal salts of these compounds have greater water solubility, and if introduced, they would exist primarily in the dissolved form. The tendency of chlorophenols to ionize depends on the pH of the system. They are nonionized in aqueous solutions with pH lower than 5 and become increasingly dissociated as the pH rises. The degree of dissociation could determine the extent of sorption of trichlorophenols and tetrachlorophenols by colloids in aquatic systems; however, specific information is not available. Hydrological factors such as current patterns and mixing as well as sorption, degradation, and migration of organisms affect the movement of these chemicals.

There is limited evidence which indicates the microbiological degradation of 2,4,5- and 2,4,6-trichlorophenol in aquatic environments. Mixed microbial populations found in activated sludge and waste lagoons degrade these chemicals readily (Ingols, Gaffney, and Stevenson, 1966; Sidwell, 1971).

C.2.5.3 Soils

The movement of trichlorophenols and tetrachlorophenols in soils depends largely on the interaction and persistence of the chemicals. The extent of sorption determines whether the chemicals are carried in association with eroded soils or move through the soil profile with water. Sorption characteristics of trichlorophenols and tetrachlorophenols have not been studied. Due to their weakly acidic properties and low water solubilities, they may be sorbed substantially by soil colloids, particularly the organic colloids.

Available data indicate that microorganisms play an important role in the dissipation of trichlorophenols and tetrachlorophenols in the environment. Bacterial species capable of metabolizing 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol have been isolated from soils and activated sludge (Tabak, Chambers, and Kabler, 1964; Nachtigall and Butler, 1974). In addition, certain species of fungi and mixed bacterial populations found in broiler house litters can degrade 2,3,4,6-tetrachlorophenol (Gee and Peel, 1974). This degradation may be an important removal mechanism for trichlorophenols and tetrachlorophenols present in wood shavings used for litter.

Persistence of trichlorophenols and tetrachlorophenols appears to depend on the position of the chlorine atoms on the aromatic ring. Isomers with chlorine in the meta position seem to degrade less readily in soils (Alexander and Aleem, 1961), indicating that 2,4,5-trichlorophenol and the tetrachlorophenol isomers persist longer in the environment. In contrast, Ide et al. (1972) found rapid disappearance of tetrachlorophenols in flooded soils, with 2,3,4,6-tetrachlorophenol degrading the fastest. Factors affecting the persistence of these chlorophenols need further study.

C.2.6 ANALYSIS

C.2.6.1 Sampling, Storage, and Preservation

Sample collection, handling, and storage techniques for trichlorophenols and tetrachlorophenols are essentially the same as those described for 2-chlorophenol and 2,4-dichlorophenol in Section B.2.6.1.

C.2.6.2 Sample Preparation for Analysis

The methods of preparation used for samples to be analyzed for trichlorophenols and tetrachlorophenols are the same as those used for 2-chlorophenol and 2,4-dichlorophenol (Section B.2.6.2).

C.2.6.3 Determination and Identification

The methods for detection of trichlorophenols and tetrachlorophenols in environmental samples are the same as those used for 2-chlorophenol and 2,4-dichlorophenol: colorimetry, chromatography, ultraviolet and infrared spectrophotometry, and mass spectrometry (Table C.2.1). See

TABLE C.2.1. METHODS OF DETERMINATION OF 2,4,5-TRICHLOROPHENOL, 2,3,4,6-TETRACHLOROPHENOL, AND [illegible]

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
Aqueous solution of 2-chlorophenol, 2,4-dichlorophenol, and 2,4,5-trichlorophenol	Samples extracted with hexane and dansyl derivatives of chlorophenols separated by thin-layer chromatography	Fluorescence spectrometry of dansyl derivatives	In situ measurement of fluorescence on thin-layer chromatographic plates; confirmation analysis by [illegible] mass spectrometry	[illegible] and [illegible], [illegible]
2,3,4,6-Tetrachlorophenol in fats, oils, and fatty acids	5-g samples treated with H_2SO_4 and Celite, followed by liquid-liquid extractions with petroleum ether, aqueous alkali, and $CHCl_3$	Gas-liquid chromatography using electron-capture detector at level of [illegible] µg/ml	[illegible]	[illegible]
2,4-Dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol in natural waters	1000-ml acidified samples extracted with petroleum ether	Two-dimensional thin-layer chromatography at 1 µg/liter	[illegible]	[illegible]
2,4,6-Trichlorophenol and 2,3,4,6-tetrachlorophenol in mixtures of standard chlorophenols	Phenols methylated with diazomethane	Flame-ionization gas-liquid chromatography	[illegible]	[illegible]
2-Chlorophenol, 2,4-dichlorophenol, and 2,3,4,5-tetrachlorophenol in mixtures of standard phenols	Phenols separated on a polyacrylate resin column	High-pressure liquid chromatography with UV detector	[illegible]	[illegible]
2,3,4,6-Tetrachlorophenol in wood shavings and chicken tissues	200 g of wood shavings or [illegible] g of chicken tissue steam distilled; distillate extracted with pentane and extract ethylated with diazoethane	Gas-liquid chromatography using electron-capture detector at [illegible] for wood and [illegible] for chicken tissue	[illegible]	[illegible]
2-Chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol in mixtures of standard chlorophenols		Gas-liquid chromatography	[illegible]	[illegible]

(continued)

TABLE [illegible] (continued)

Sample	Isolation method	Analytical method and conditions	[illegible]	[illegible]
2,4,6-Trichlorophenol and 2,3,4,6-tetrachlorophenol in organic tissue	10-g sample homogenized with a mixture of hexane and acetone, followed by extraction with a mixture of acidified hexane and diethyl ether; compounds sorbed by an anion-exchange resin and resin extracted with benzene; extract methylated with diazomethane	[illegible]	[illegible]	[illegible]
2,4,6-Trichlorophenol and 2,3,4,6-tetrachlorophenol in soil	10 g of soil extracted with NaOH; extract shaken with anion-exchange resin and resin extracted with benzene; derivatives formed with diazomethane	[illegible]	[illegible]	[illegible]
2,4,6-Trichlorophenol and 2,3,4,6-tetrachlorophenol in water	1000 ml of water percolated through an anion-exchange resin; resin extracted with benzene; derivatives formed with diazomethane	[illegible]	[illegible]	[illegible]
Tetrachlorophenol in air	Air passed through alkali absorbing solution and treated with potassium ferricyanide and 4-aminoantipyrine	[illegible]		[illegible]
Tetrachlorophenol in human urine	Acidified sample distilled, followed by extraction of distillate with xylene	[illegible]		[illegible]
2,4-Dichlorophenol and 2,4,5-trichlorophenol in rat urine	1- to 5-ml samples hydrolyzed with H_2SO_4 followed by extraction with ethyl ether and ethylation with diazoethane; eluted from silica gel column with benzene-hexane	[illegible]	[illegible]	[illegible]
2,4-Dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol, and 2,3,4,5- and 2,3,4,6-tetrachlorophenol in rabbit urine	Extracted with diethyl ether, concentrated by distillation; eluted from silica gel column successively with hexane and benzene in hexane; eluate extracted with NaOH	[illegible]	[illegible]	[illegible]

TABLE C.2.1 (continued)

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
2,4-Dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol in mouse urine	Partitioned into benzene and water; phenols determined in both phases	Thermal-conductivity gas-liquid chromatography	Thin-layer chromatography used for purification of compounds; enzyme hydrolysis of water-soluble chlorophenol conjugates; confirmation by mass fragmentography	Kurihara and Nakajima, 1974
2,4-Dichlorophenol and 2,4,5-trichlorophenol in milk and cream	10-g acidified sample extracted with diethyl ether, eluted through alumina column with diethyl ether, followed by series of extractions of eluate with dilute NaOH and benzene	Gas-liquid chromatography using microcoulometric or electron-capture detector at levels of 0.05 µg/ml	Column chromatography for cleanup; NaOH or benzene extraction of eluate unnecessary for 2,4,5-trichlorophenol; recovery >80%	Bjerke et al., 1972
2,4-Dichlorophenol and 2,4,5-trichlorophenol in sheep and cattle tissues	5-g sample acid hydrolyzed with or without prior alkaline digestion; codistilled with water followed by partitioning into methylene chloride formation of trimethylsilyl ether	Gas-liquid chromatography at levels of 0.01 to 0.02 µg/g	Distillation for cleanup; no interference of coextractives; alkaline digestion increases recovery; recovery >95%	Clark et al., 1975
2-Chlorophenol, 2,4-dichlorophenol, and 2,4,5- and 2,4,6-trichlorophenol in water	Chlorophenols absorbed on activated carbon, adsorbates removed by stepwise extraction with $CHCl_3$, NaOH, and diethyl ether	Gas-liquid chromatography	Florisil column for cleanup	Eichelberger, Dressman, and Longbottom, 1970
2-Chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol in water	Sample used to prepare 4-aminoantipyrine and p-nitrophenylazo dye derivatives and extracted with $CHCl_3$ or ether	Thin-layer chromatography		Aly, 1968

Section B.2.6.3 for discussions of the 4-aminoantipyrine colorimetric method and thin-layer chromatography.

Gas-liquid chromatography, the most widely used technique for separating, identifying, and quantifying chlorophenols in various kinds of samples, employs either electron-capture or flame-ionization detectors (Table C.2.1). Because gas-liquid chromatography is sensitive to nanogram levels, it is adaptable for the quantitation of trace amounts of chlorophenols. Although thin-layer chromatography can resolve chlorophenols satisfactorily, it is, at best, semiquantitative. Gas-liquid chromatography has been used for analysis of water and wastewater containing mixtures of chlorophenol isomers after extraction and cleanup of samples (Eichelberger, Dressman, and Longbottom 1970; Sidwell, 1971; Manufacturing Chemists Association, 1972). The order of elution of isomers resulting from the chlorination of phenols in water (Manufacturing Chemists Association, 1972) and in chlorophenolic wastewater (Sidwell, 1971) is 2-chlorophenol, phenol, 2,6-dichlorophenol, 2,5-dichlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4-chlorophenol, and 2,4,5-trichlorophenol. 2,4,5-Trichlorophenol and/or its 2,4,6- isomer have been identified as major degradation products in the metabolism of lindane (hexachlorocyclohexane) in rabbits (Karapally, Saha, and Lee, 1973), rats (Freal and Chadwick, 1973), and mice (Kurihara and Nakajima, 1974). Gas-liquid chromatography has also been utilized successfully in identifying 2,4,5-trichlorophenol as the prime metabolite of the mammalian metabolism of the pesticides 2,4,5-T (Bjerke et al., 1972; Clark et al., 1975) and ronnel (Shafik, Sullivan, and Enos, 1973) and in identifying 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol in oils and fatty acids (Higginbotham, Ress, and Rocke, 1970), in fortified biological tissues, and in soils (Gee, Land, and Robinson, 1974; Renberg, 1974). The degradation products of pentachlorophenol in rice soils consisted mainly of tetrachlorophenol and some trichlorophenol isomers and were quantified by gas chromatography (Ide et al., 1972; Kuwatsuka and Igarashi, 1975).

Biological samples such as animal tissues and soils require more extensive cleanup than water samples before they are suitable for gas-liquid chromatography. Problems of poor resolution and tailing encountered on samples can be corrected by judicious selection of columns and type of derivatization (Barry, Vasishth, and Shelton, 1962; Kilgore and White, 1970; Karapally, Saha, and Lee, 1973; Shafik, Sullivan, and Enos, 1973; Svec and Zbirovsky, 1974; Gee, Land, and Robinson, 1974; Kurihara and Nakajima, 1974; Renberg, 1974). This is an important prerequisite because isomers may have the same retention time. For example 2,4,5- and 2,4,6-trichlorophenol and 2,3,5,6-tetrachlorophenol have the same retention time on 15% OV-17 and 5% QF-1 columns (Kilgore and White, 1970; Karapally, Saha, and Lee, 1973). Both 2,3,4,6- and 2,3,4,5-tetrachlorophenol have the same retention time on a 15% OV-17 column (Karapally, Saha, and Lee, 1973); however, their methyl ether (anisole) derivatives, formed by reacting the isomers with diazomethane, are separated easily on the same column. Svec and Zbirovsky (1974) claimed that the anisole derivatives were more volatile than the free chlorophenols and that the chromatographic peaks were sharp and symmetrical. Methylation using dimethyl sulfate (Ide et al., 1972) and ethylation with diazomethane

(Shafik, Sullivan, and Enos, 1973; Gee, Land, and Robinson, 1974) also improve the resolution and identification of trichlorophenols and tetrachlorophenols (Shafik, Sullivan, and Enos, 1973; Gee, Land, and Robinson, 1974). Use of dual columns could improve the separation of the higher chlorinated isomers, as suggested by Barry, Vasishth, and Shelton (1962). Columns reported to be satisfactory for the segregation of the anisole derivatives of trichlorophenols and tetrachlorophenols include 5% QF-1 on 80/100-mesh Chromaton N-HMDS-AW (Svec and Zbirovsky, 1974), 5% QF-1 on 70/80-mesh Chromosorb G-DMCS-AW (Kilgore and White, 1970), 15% OV-17 on Chromosorb W (Karapally et al., 1973), 10% Carbowax 20M or 10% Apiezon L on 80/100-mesh CQ Celite (Gee, Land, and Robinson, 1974), and 8% QF-1 and 4% SF-96 on 100/120-mesh Chromosorb W (Renberg, 1974), 4% SE-30 and 6% QF-1 on 80/100-mesh Chromosorb W-HP (Shafik, Sullivan, and Enos, 1973), and 1.5% neopenthyl glycol succinate on 60/80-mesh Chromosorb W-AW (Kurihara and Nakajima, 1974). Separation of 2,4,6- and 2,4,5-trichlorophenol from extracts of water, milk, and animal tissues is accomplished by using 15% K20M Carbowax TPA on 80/100-mesh Chromosorb W-HMDS (Manufacturing Chemists Association, 1972), 1.5% FFAP (free fatty acid phase) on 100/120-mesh Chromosorb G-AW-DMCS (Sidwell, 1971), 3% OV-1 on 80/90-mesh Chromosorb W (Clark et al., 1975), and 4% LAC-446 and 0.55% phosphoric acid on 80/100-mesh Chromosorb W-HP (Bjerke et al., 1972). 2,3,4,6-Tetrachlorophenol can be separated from oils and fats by using 10% diethylene glycol succinate and 2% phosphoric acid on a 60/80-mesh Gas Chrom P column (Higginbotham, Ress, and Rocke, 1970). 2,4,6-Trichlorophenol and 2,3,5,6-, 2,3,4,6-, and 2,3,4,5-tetrachlorophenol can be separated from rice soils with 1% DEGS and 1% phosphoric acid on Chromosorb W (Kuwatsuka and Igarashi, 1975).

Ultraviolet and infrared spectrophotometry have also been used to identify trichlorophenols and tetrachlorophenols (Burttschell et al., 1959; Barry, Vasishth, and Shelton, 1962). However, these methods are less sensitive than gas-liquid chromatography and are probably more useful for confirmatory tests than for quantitative analysis. Mass spectrometry is also used for confirmatory analysis (Ide et al., 1972; Frei-Häusler, Frei, and Hutzinger, 1973; Karapally, Saha, and Lee, 1973; Kurihara and Nakajima, 1974; Safe, Jamieson, and Hutzinger, 1974; Svec and Zbirovsky, 1974), but this technique is complex and expensive. All of these methods require prior separation of the compound of interest before identification is made.

SECTION C.2

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C.3 BIOLOGICAL ASPECTS IN MICROORGANISMS

C.3.1 BACTERIA

C.3.1.1 Metabolism

The microbial degradation of trichlorophenols and tetrachlorophenols is extensively discussed in Section C.7; therefore, only a brief overview is presented in this section. Microbial degradation of trichlorophenols and tetrachlorophenols has been examined in soils, activated sludge, lagoon effluent, and enrichment cultures. Soil microorganisms capable of metabolizing trichlorophenols and tetrachlorophenols have not been identified. A *Pseudomonas* sp. isolated from activated sludge was capable of oxidizing 2,4,6-trichlorophenol (Nachtigall and Butler, 1974). Metabolic pathways were not determined. An activated sludge culture acclimated to phenol was able to metabolize 2,4,6-trichlorophenol (Chambers, Tabak, and Kabler, 1963; Tabak, Chambers, and Kabler, 1964). Predominant organisms in the mixed bacterial cultures were *Pseudomonas* sp., *Achromobacter* sp., and *Flavobacterium* sp. A gram-variable bacillus designated as KC-3 (derived from a pentachlorophenol enrichment culture) was capable of metabolizing 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol (Chu, 1972). It is not known if bacteria capable of metabolizing the higher phenols are widespread in the environment; more research in this area is warranted. The pathways and mechanisms of microbial metabolism have not been elucidated for trichlorophenols and tetrachlorophenols. Dechlorination, hydroxylation, and ring cleavage may be involved in the process.

Gee and Peel (1974) studied chlorophenol metabolism by mixed bacterial populations isolated from broiler house litter. A mixed bacterial suspension obtained from broiler house litter was able to metabolize 2,3,4,6-tetrachlorophenol completely within five days without the accompanying production of 2,3,4,6-tetrachloroanisole. No chlorophenol metabolism was seen when the bacteria were cultured under anaerobic conditions.

C.3.1.2 Effects

The mechanism of toxicity of higher chlorophenols to bacteria is not completely understood. In general, the mode of antibacterial action of phenolic compounds involves several steps: (1) adsorption to the bacterial cell wall, (2) inactivation of essential enzymes, and (3) finally, lysis and death of the cell (Klarmann, 1963). Phenolic compounds bind and denature proteins and at high concentrations are likely to act as gross protoplasmic poisons which penetrate rapidly and rupture the cell wall. However, chlorophenols are frequently toxic at levels too low to denature proteins. Thus, another mechanism must be operative at these concentration levels. An effect on cell membranes — with subsequent leakage of cell constituents and perhaps inactivation of specific enzyme systems — has occurred following exposure of bacteria to chlorophenols (Goldacre and Galston, 1953; Judis, 1962, 1963). Because higher chlorophenols have low water solubility and high lipid solubility, their gross effect on bacteria is probably due to association with cellular lipids and subsequent alteration of cell permeability (Baker, Schumacher, and Roman, 1970). Several studies have dealt with the toxicological effect of 2,4,6-trichlorophenol on bacteria, but investigations specifically addressing the toxicity mechanisms of 2,4,5-trichlorophenol or tetrachlorophenols were not found. In general, the mechanism of toxicity of these compounds may resemble that of 2,4,6-trichlorophenol.

Judis (1962) observed the leakage of ^{14}C-labeled glutamate from *Escherichia coli* cells exposed to 2,4,6-trichlorophenol. The *E. coli* cells with ^{14}C-labeled glutamate lost free glutamate, and the label was incorporated into other nondiffusible compounds. Thus, leakage from the cell, if detected, implies injury to the cell membrane integrity or to the selective permeability mechanisms within the cell membrane. Cells incubated for 10 min at 22°C in a solution of 2,4,6-trichlorophenol (333 mg/liter) released ten times more radioactivity than control cells. The author concluded that 2,4,6-trichlorophenol may exert its lethal activity by damaging the cell membrane or systems which control membrane permeability. In another investigation, Judis (1963) determined the effect of 2,4,6-trichlorophenol on glucose and sodium sulfate leakage from *E. coli*. No leakage of ^{14}C-labeled glucose or ^{35}S-labeled sodium sulfate was seen at 2,4,6-trichlorophenol concentrations of 167 or 333 mg/liter, but a pronounced leakage of ^{14}C-labeled glutamate was found; this finding was consistent with the previous study. Thus, although 2,4,6-trichlorophenol exposure resulted in the leakage of glutamate from treated cells, thereby implying membrane damage, at least two other compounds did not leak in a similar manner. The mechanism of this selective effect on cell membrane permeability for different molecules is not known. The contribution of leakage of glutamate (and possibly other cell constituents) to the toxicity of 2,4,6-trichlorophenol to bacteria is not known, and further research is warranted.

Goldacre and Galston (1953) demonstrated an effect of 2,4,6-trichlorophenol on the in vitro activity of crystalline beef liver catalase. 2,4,6-trichlorophenol, at a concentration of 10^{-2} *M* (2 g/liter), inhibited the catalase reaction by 50%. This effect was the weakest among six chlorophenol compounds tested in the system. The investigators suggested that this effect on the catalase system may be responsible for some bactericidal action of chlorophenols, but additional data are needed.

All chlorophenols possess some bactericidal properties (Klarmann, 1963; Skyes, 1965; Baker, Schumacher, and Roman, 1970). Antibacterial effectiveness generally increases with the degree of chlorine substitution up to the trichloro derivatives. The tetrachloro isomers are considerably less active than the trichloro isomers, and pentachlorophenol is even less active. However, some of the higher chlorophenols, particularly 2,3,4,6-tetrachlorophenol and pentachlorophenol, possess enhanced fungicidal activity. The bactericidal action of disinfectants is frequently expressed in terms of the phenol coefficient (Section B.3.1).

2,4,5-Trichlorophenol is produced in large quantities in the United States, primarily as a precursor compound in the manufacture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The compound is available commercially under several trademark designations, and both 2,4,5-trichlorophenol and its sodium salt are sold in flake form for germicidal use (Doedens, 1963). The sodium salt of 2,4,5-trichlorophenol is marketed by Dow Chemical Corporation under the trade name Dowicide B. Antibacterial efficiencies (expressed as a concentration necessary for growth inhibition) for the higher chlorophenols are presented in Table A.3.1.

2,4,6-Trichlorophenol is sold commercially by a number of producers. It is used as a bactericide, a fungicide, a general antiseptic, a wood and glue preservative, an insecticide ingredient, and an antimildew treatment for textiles (Doedens, 1963). Although 2,4,6-trichlorophenol is recognized as an effective microbicide, toxicity data for various species of bacteria are scant. Sykes (1965) reported the bactericidal activity of 2,4,6-trichlorophenol in strains of *Salmonella typhi* and *Staphylococcus aureus*. The phenol coefficients were 23.0 and 25.0 respectively. Thus, 2,4,6-trichlorophenol is approximately 24 times more effective against these two bacterial strains than phenol.

The pH of the medium has a profound effect on the bactericidal efficiency of chlorophenols (Howard and Durkin, 1973). Ordal (1941) noted that 2,4,6-trichlorophenol was far more effective in inhibiting the growth of *Staphylococcus aureus* at low pH. At pH 5.8, levels of 500 mg/liter 2,4,6-trichlorophenol inhibited completely the growth of the bacterium in culture medium. On the other hand, almost 30 g/liter was required for complete inhibition at pH 9.8. The author noted that because the p*K* value of 2,4,6-trichlorophenol is 6.2, the undissociated compound is far more germicidal to *Staphylococcus aureus* than is the dissociated compound. A linear relationship exists between the logarithms of the LC_{50} values and the solubility of the higher chlorophenols (Blackman, Parke, and Garton, 1955). These data seem to indicate that the toxic effect of the chlorophenols is increased as the pH of the medium approaches the p*K* of the chlorophenols. Thus, the effect of pH on toxicity must be considered when data from different laboratories and investigators are compared.

2,4,6-Trichlorophenol may pose a hazard in sewage treatment facilities. The Manufacturing Chemists Association (1972) reported that 2,4,6-trichlorophenol caused a significant inhibition of oxygen uptake in a mixed microbial population. Inhibition was detected in a synthetic sewage preparation at 2,4,6-trichlorophenol concentrations of 50 and 100 mg/liter; no effect was noted at 1 or 10 mg/liter. Although this experiment indicates that the compound may be capable of interfering with sewage treatment processes, no other data directly addressing the effect of 2,4,6-trichlorophenol on sewage treatment works are available; further investigations are warranted.

Among the tetrachlorophenol isomers, only 2,3,4,6-tetrachlorophenol is of any commercial importance. The chief applications of the compound and its salts are as bactericides for latex preservation, wood preservatives, insecticides, and leather preservatives (Doedens, 1963).

C.3.2 FUNGI

C.3.2.1 Metabolism

Information on metabolism of 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol by fungi is derived primarily from studies of musty taint in chicken and chicken eggs (Section C.5.1.2.2.3). Musty taint appears in chickens following methylation of 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol by fungi in poultry litter and subsequent absorption of the anisoles by the chickens. Many species of fungi commonly present in poultry litter are capable of methylating chlorophenols to their corresponding anisoles. Methylation of 2,4,6-trichlorophenol to 2,4,6-trichloroanisole was demonstrated by Lane et al. (1975). Fungal species such as *Aspergillus sydowi*, *Scopulariopsis brevicaulis*, and *Penicillium crustosum* were capable of catalyzing the methylation reaction, but mechanisms for the reaction were not determined.

Efficient methylation of 2,3,4,6-tetrachlorophenol by a number of fungal species present in poultry litter was reported by Curtis et al. (1972, 1974). Gee and Peel (1974) screened 26 fungal species for ability to metabolize and methylate 2,3,4,6-tetrachlorophenol over a five-day period. The experiments were designed to determine if the ability to metabolize chlorophenols is widespread among litter microorganisms and the extent to which metabolism is associated with methylation. Gee and Peel used 116 isolates of 26 fungal species found in poultry litter; 99 of the 116 isolates tested were capable of metabolizing chlorophenol (assayed by disappearance of the compound from the culture medium) and 68 of them produced 2,3,4,6-tetrachloroanisole (as assayed by the appearance of the compound in the medium). Some of the isolates metabolized most of the chlorophenol but did not produce 2,3,4,6-tetrachloroanisole (e.g., *Penicillium brevi-compactum*). This information suggests that two different pathways exist for the utilization of 2,3,4,6-tetrachlorophenol by fungi and that only one involves methylation to the chloroanisole (Table C.3.1). The percentage of 2,3,4,6-tetrachlorophenol methylated differed widely among isolates, even isolates of the same species. The greatest methylation was observed with *Penicillium corylophilum*.

C.3.2.2 Effects

The antifungal activities of 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol have been documented; their toxicities to various species of fungi are presented in Table A.3.3. Information on the mechanism of toxic action of higher chlorophenols to fungi is not available; toxicity may result from mechanisms similar to those discussed for bacteria (Section C.3.1.2). Reported effects on oxidative phosphorylation (Section C.6.2.2.1) may also be important in the toxicity of these chlorophenols to fungi.

TABLE C.3.1. METABOLISM OF 2,3,4,6-TETRACHLOROPHENOL BY FUNGAL ISOLATES

Fungal species	Isolate	2,3,4,6-Tetrachlorophenol Metabolized[b] (%)	2,3,4,6-Tetrachlorophenol Methylated (%)
Absidia cylindrospora	CD5	9	0
	CD119	0	0
	UD323	0	0
Aspergillus amstelodami	US90	95	39
	US11	26	2
	US196	25	3
	US28	8	Trace
A. candidus	US177a	72	Trace
	M29[b]	45	Trace
	US189	15	0
	US46	14	0
	S44[b]	10	0
	T3[b]	0	0
	US78b	0	Trace
	M33[b]	0	0
A. chevalieri	US66	27	3
	US52	19	1
	US10	12	1
	US63	9	0
	US50	0	Trace
A. chevalieri var. *intermedius*	US13	100	45
	US264	32	Trace
A. clavatus	F61[b]	16	6
	F44[b]	13	2
	F68[b]	13	2
A. flavus	US71	0	Trace
	US67	0	Trace
	US222	0	Trace
	US77	0	0
A. flavus var. *columnaris*	UD33	4	0
	UD215	0	0
A. niger	CS14	35	2
	UD314	31	3
	CS142	17	4
	CS180	14	0
	L9[b]	4	8
	L10[b]	0	3

(continued)

TABLE C.3.1 (continued)

Fungal species	Isolate	2,3,4,6-Tetrachlorophenol	
		Metabolized[a] (%)	Methylated (%)
A. petrakii	UD130	99	40
	UD51	88	44
A. repens	US35	99	48
	S51[b]	99	61
	US31	99	61
	US21	99	25
	T24[b]	97	74
	US142	97	45
	M40[b]	99	64
	US22	35	27
A. restrictus	US12	14	0
A. ruber	US98	100	30
	L14[b]	92	31
	US43	32	3
	US91	28	0
	L13[b]	24	8
	US97	18	Trace
	US153	8	Trace
A. sydowi	Q9[b]	100	74
	US194	100	27
	S42[b]	97	48
	M38[b]	95	42
	US135	92	22
	S37[b]	87	56
	US15	83	62
	US103	27	10
	T21[b]	22	17
A. versicolor	US184	98	42
	US61	89	34
	US120	81	80
	US228	69	18
	M37[b]	47	6
	M35[b]	37	19
	S60[b]	9	7
A. wentii	CS112	30	0
	UD271	4	Trace[c]
Mucor racemosus	CD56	0	0
	US100	0	0

(continued)

TABLE C.3.1 (continued)

Fungal species	Isolate	2,3,4,6-Tetrachlorophenol	
		Metabolized[a] (%)	Methylated (%)
Paecilomyces varioti	CS18	100	81
	CS21	69	56
	CS84	34	14
	CS11	16	11
Penicillium brevi-compactum	US123	100	Trace
	CS121b	97	Trace
	US62	28	0
	CS109	0	Trace
P. chrysogenum	US198	52	0
	US49	40	Trace
	US75	35	Trace
	US72	15	0
P. corylophilum	US113	98	80
	US162	97	62
	US160	96	83
	US117	95	75
P. crustosum	US59	71	2
	T12[b]	57	3
	R13[b]	47	Trace
	US201	46	16
	US185	41	7
	L3[b]	38	14
	S33[b]	25	6
	US129	16	Trace
P. frequentans	US52	86	54
	CS127	30	Trace
	US24	22	0
	US30	4	0
P. roqueforti	CS121a	99	65
	CS126	72	27
	CS129	53	8
	CS158	12	7
Rhizopus oryzae	CS97	55	Trace
	CS155	0	0
Scopulariopsis brevicaulis	D6[b]	87	60
	US178	52	6
	US17	23	1
	US1	20	Trace

(continued)

TABLE C.3.1 (continued)

Fungal species	Isolate	2,3,4,6-Tetrachlorophenol	
		Metabolized[a] (%)	Methylated (%)
	US171	15	Trace
	T8[b]	6	1
	M7[b]	0	Trace

[a] Taken as the difference between residual chlorophenol in treated cultures and that found in uninoculated control cultures.

[b] Isolates obtained from litter associated with musty chickens.

[c] A definite trace but <1%.

Source: Adapted from Gee and Peel, 1974, Table I, pp. 240-241. Reprinted by permission of the publisher.

C.3.3 ALGAE

C.3.3.1 Metabolism

No information is available on the metabolism of 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, or tetrachlorophenols by algae. Algae may be capable of assimilating and metabolizing chlorophenols in the environment, but further investigation is warranted.

C.3.3.2 Effects

As discussed previously (Section B.3.3.2), the healthy growth of algae in waste stabilization ponds is important for reoxygenation purposes, and considerable concern has been expressed over the toxic effects of organic chemicals on the photosynthetic activity of algae. The effects of chlorophenols on the synthesis of chlorophyll by the blue-green algae *Chlorella pyrenoidosa* under laboratory conditions were investigated by Huang and Gloyna (1967). They found that 2,4,5- and 2,4,6-trichlorophenol possess similar toxicological properties; both compounds seriously affect chlorophyll synthesis at concentrations greater than 2.5 mg/liter (Table A.3.4). Both trichlorophenol isomers are far more toxic to *Chlorella* than either 2,4-dichlorophenol or the monochlorophenols. At a concentration of 1.0 mg/liter, 2,4,5- and 2,4,6-trichlorophenol showed a minimal effect on chlorophyll synthesis; this value was accepted as a minimum toxic level. Huang and Gloyna (1967) concluded that increasing the number of chlorine atoms on the aromatic ring of chlorophenols enhanced their toxicity. Both 2,4,5- and 2,4,6-trichlorophenol may pose a serious threat to algae in waste stabilization ponds. Care must be taken that these compounds are not dumped or allowed to seep into such waste treatment facilities.

SECTION C.3

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C.4 BIOLOGICAL ASPECTS IN PLANTS

C.4.1 METABOLISM

C.4.1.1 Uptake and Absorption

No reports on the direct uptake and absorption of 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols were found. Kohli, Weisgerber, and Klein (1976) reported the formation of 2,3,4,6-tetrachlorophenol in lettuce plants following treatment with lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane). Lettuce plants (approximately three weeks old) were planted in nutrient solution containing ^{14}C-labeled lindane at a level of 1.45 mg/liter. Plants were grown in flasks for four weeks, and at the end of the treatment both the plants and the nutrient solution were tested for the presence of lindane and its metabolic products. Of the original radioactivity, 7.8% was found in the nutrient solution at the end of the four-week treatment; 1% or less of the 7.8% was present as 2,3,4,6-tetrachlorophenol. No conjugates of 2,3,4,6-tetrachlorophenol were detected in the nutrient solution. Analysis of the plants revealed the presence of ^{14}C-labeled compounds comprising 14.1% of the original radioactivity in the nutrient solution. Both 2,3,4,6-tetrachlorophenol and tetrachlorophenol conjugates were detected in the plants. Approximately 1% of the radioactivity found in the plants was identified as free 2,3,4,6-tetrachlorophenol, and 3% consisted of tetrachlorophenol conjugates. The chemical nature of the conjugates was not determined. The metabolic pathways responsible for the presence of 2,3,4,6-tetrachlorophenol in plants treated with lindane were not discussed.

C.4.1.2 Transport and Distribution

No information on the transport and distribution of 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols was found. The presence of 2,3,4,6-tetrachlorophenol in lettuce plants grown in a nutrient solution containing lindane (Section C.4.1.1) resulted from uptake of lindane and its subsequent biodegradation to 2,3,4,6-tetrachlorophenol in the plant (Kohli, Weisgerber, and Klein, 1976). However, tetrachlorophenol could possibly have formed in the nutrient solution with subsequent direct plant uptake. Unfortunately, this study sheds little light on the transport and distribution of 2,3,4,6-tetrachlorophenol in plants.

C.4.1.3 Biotransformation

No information on the biotransformation of 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols was found. Kohli, Weisgerber, and Klein (1976) found 2,3,4,6-tetrachlorophenol conjugates as well as free 2,3,4,6-tetrachlorophenol in lettuce plants (Section C.4.1.1). The nature of these conjugates and their role in the biodegradation of 2,3,4,6-tetrachlorophenol are unknown.

C.4.1.4 Elimination

No information was found on the elimination of trichlorophenols or tetrachlorophenols in vascular plants.

C.4.2 EFFECTS

C.4.2.1 Physiological or Biochemical Role

No physiological role or requirement for trichlorophenols or tetrachlorophenols in plants has been reported.

C.4.2.2 Toxicity

C.4.2.2.1 Mechanism of Action — No information on the mechanism of toxicity of trichlorophenols or tetrachlorophenols to vascular plants was located.

C.4.2.2.2 General Toxicity — Blackman, Parke, and Garton (1955) determined the concentrations of 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol in water which caused "death" in half of the fronds of the aquatic plant *Lemna minor*. *Lemna minor* plants were grown in nutrient solution containing various concentrations of these chlorophenols for 48 hr. Plants were grown at pH 5.1 with illumination of 320 ft-c (3520 lx) and a temperature of 25°C. At the end of the 48-hr treatment, the fronds were removed, washed with distilled water, and transferred to chlorophenol-free nutrient solution for another 24 hr. At the end of the treatment the degree of chlorosis was recorded. Injury to fronds was assessed by assuming that those fronds that were colorless on move than half of their surfaces were counted as "dead." The concentration of any chlorophenol which caused "death" in half the fronds was calculated. These LC_{50} values for 2,3,4,5-tetrachlorophenol, 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol were calculated as 2.6×10^{-6} *M*, 8.4×10^{-6} *M*, and 3×10^{-5} *M* respectively. Thus, under these experimental conditions, 2,3,4,5-tetrachlorophenol was approximately three times more toxic to *Lemna minor* than was 2,4,5-trichlorophenol, and 2,4,5-trichlorophenol was three times more toxic than 2,4,6-trichlorophenol. These values were corrected for the effects of dissociation of the compounds at pH 5.1 because the p*K* values for the compounds vary over a considerable range. In another study, no effect was seen on *Ludvigia* sp. or *Anacharis* sp. over an eight-month period in a flow-through bioassay system at 2,4,6-trichlorophenol concentrations from 0.01 to 4.1 mg/liter (Manufacturing Chemists Association, 1972).

C.4.2.2.3 Mitotic Effects — Amer and Ali (1974) investigated the mitotic effect of 2,4,5-trichlorophenol on *Vicia faba*. Plants were sprayed daily with 7 ml of aqueous 2,4,5-trichlorophenol solution containing 63 mg trichlorophenol per liter. Flower buds were gathered either 5 or 25 days following initiation of treatment, and pollen mother cells were checked for mitotic abnormalities. Abnormalities were not detected in plants treated with 2,4,5-trichlorophenol, but significant increases in mitotic abnormalities were seen in plants sprayed with a solution containing 0.39 mg 2,4-dichlorophenol per liter (Section B.4.2.2.3). No information regarding possible mitotic effects of 2,4,6-trichlorophenol or tetrachlorophenols was found.

SECTION C.4

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C.5 BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

C.5.1 BIOLOGICAL ASPECTS IN BIRDS AND MAMMALS

Data on the effects of 2,4,5- and 2,4,6-trichlorophenol and tetrachlorophenols on mammals primarily involve experimental animals and are therefore discussed in Section C.6. This section summarizes the effects of these compounds on wild and domestic mammals.

C.5.1.1 Metabolism

C.5.1.1.1 Uptake and Absorption - No studies have addressed the direct uptake of higher chlorophenols in birds and domestic mammals. 2,4,5-Trichlorophenol may appear in domestic animals exposed to chemically similar substances. For example, ronnel [*O*,*O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate] is an organophosphate used extensively for the control of cattle grubs and external parasites such as lice, flies, and ticks. It also is used as a systemic grub control agent and has been sold in the form of medicated mineral mixtures or concentrates which are either fed directly or are mixed with feed ingredients (Gehrt, 1972). The chemical structure of ronnel suggests that 2,4,5-trichlorophenol may be a metabolic breakdown product in animals, but data are not available. In addition, the presence of 10% to 20% 2,3,4,6-tetrachlorophenol in commercial pentachlorophenol provides a means of direct uptake of tetrachlorophenol by domestic animals exposed to pentachlorophenol-treated wood (Section C.5.1.2.2.3).

C.5.1.1.2 Transport and Distribution - 2,4,5-Trichlorophenol has been detected in cows fed diets containing the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Bjerke et al. (1972) studied the appearance of 2,4,5-trichlorophenol in the milk and cream of cows fed diets containing 2,4,5-T at levels of 10, 30, 100, 300, and 1000 mg/kg of feed. At the lower dose levels, cows were kept on diets containing 2,4,5-T for 14 days and then returned to normal diets without 2,4,5-T for an additional 7 days. Cows receiving 2,4,5-T at 1000 mg/kg feed were fed for 21 days, followed by 7 days on normal diets. No 2,4,5-trichlorophenol was present in the milk or cream of cows fed 2,4,5-T at the 10 or 30 mg/kg level. Demonstrable amounts of 2,4,5-trichlorophenol (as well as 2,4,5-T) were detected in the milk and cream of cows fed 2,4,5-T at levels of 1000 mg/kg in the feed (Table C.5.1). Average residues of 2,4,5-T and 2,4,5-trichlorophenol in the milk were 0.42 and 0.23 mg/liter respectively. Average residues in cream were 0.26 mg/liter 2,4,5-T and 0.19 mg/kg 2,4,5-trichlorophenol. Acid hydrolysis of the milk and subsequent residue determination indicated that no conjugation of 2,4,5-trichlorophenol had occurred. Thus, following 2,4,5-T ingestion, either 2,4,5-T or its metabolite 2,4,5-trichlorophenol must have been transported for these residues to appear in the milk. No other data on the transport and distribution of higher chlorophenols in birds or mammals are available.

TABLE C.5.1. RESIDUES OF 2,4,5-T AND 2,4,5-TRICHLOROPHENOL IN MILK AND CREAM FROM COWS FED 2,4,5-T

2,4,5-T in diet (mg/kg feed)	Time on diet (days)	Residues of 2,4,5-T (μg/liter)				Residues of 2,4,5-trichlorophenol (μg/liter)			
		Cow No. 36	Cow No. 7417	Cow No. 30	Cream composite	Cow No. 36	Cow No. 7417	Cow No. 30	Cream composite
100	2	<0.05	<0.05	<0.05					
	5	<0.05	<0.05	<0.05		0.06	0.05	<0.05	
	9	<0.05	<0.05	<0.05					
	10	<0.05	<0.05	<0.05	<0.05				0.05
	11	<0.05	<0.05	<0.05					
	12	<0.05	<0.05	<0.05	<0.05	0.05	0.07	<0.05	0.06
300	2	0.08	0.08	0.20		0.08	0.12	<0.05	
	5	<0.05	<0.05	0.08					
	9	0.05	0.06	0.28	0.10				0.09
	10	0.06	0.07	0.31	0.08				0.10
	11	<0.05	0.05	0.13	<0.05	0.09	0.13	0.16	0.12
1000	2	0.31	0.26	0.78		0.16	0.37	0.39	
	5	0.44	0.27	0.54					
	9	0.42	0.32	0.44					
	12	0.37	0.30	0.29		0.17	0.21	0.22	
	16	0.23	0.36	1.0	0.41	0.15	0.31	0.23	0.21
	17	0.33	0.28	0.75	0.25	0.18	0.23	0.21	0.17
	18	0.49	0.29	0.38	0.17				0.20
	19	0.33	0.40	0.35	0.27				0.20
	20	0.23	0.28	0.32	0.21	0.15	0.23	0.25	0.18
0[a]	1	0.07	0.12	0.12		0.09	0.22	0.13	
	3	<0.05	<0.05	<0.05		<0.05	<0.05	<0.05	
	5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
	7	<0.05	<0.05	<0.05		<0.05	<0.05	<0.05	

[a] Animals fed diets containing 1000 mg 2,4,5-T per kilogram feed for 21 days followed by a 7-day withdrawal period on 2,4,5-T-free diets.

Source: Adapted from Bjerke et al., 1972, Table III, p. 965. Reprinted by permission of the publisher.

C.5.1.1.3 Biotransformation — No reports were found on the biotransformation of trichlorophenols or tetrachlorophenols in wild and domestic animals.

C.5.1.1.4 Elimination — The available literature did not discuss the elimination of trichlorophenols and tetrachlorophenols in wild and domestic animals.

C.5.1.2 Effects

C.5.1.2.1 Physiological or Biochemical Role — No information suggested that 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols play any normal physiological role in birds or domestic animals.

C.5.1.2.2 Toxicity

C.5.1.2.2.1 Mechanisms of action — No reports discussed the mechanisms of action of these higher chlorophenols in birds or mammals.

C.5.1.2.2.2 General toxicity — Toxic effects of 2,4,5- and 2,4,6-trichlorophenol and tetrachlorophenols in birds or domestic animals have not been documented. Anderson et al. (1949) studied the effect of 2,4,5-trichlorophenol on cattle. Steers ranging in weight from 360 to 630 lb (163 to 284 kg) were fed diets containing the zinc salt of 2,4,5-trichlorophenol. Animals were given daily doses of either 18 mg/kg or 159 mg/kg for 78 days or 53 mg/kg for 154 days. No effect was seen on packed blood cell volume, nor was hemoglobin affected by the treatments. No abnormalities were noted in the organs or other tissues of slaughtered animals, and the percent gain in weight and in feed consumption was approximately the same in the treated animals as in the controls.

C.5.1.2.2.3 Musty taint — An economically important problem has recently surfaced in the broiler chicken industry in England — the presence of musty taint in chickens and their eggs. Curtis et al. (1972, 1974) found that this musty taint is caused by 2,3,4,6-tetrachloroanisole and pentachloroanisole in broiler chickens. The primary source of musty taint appears to be 2,3,4,6-tetrachloroanisole. Substantial quantities of anisoles are found in wood shavings used as bedding materials in the chicken cages. Anisoles apparently are formed in the litter as a result of methylation of the parent chlorophenols by fungi present in the litter. Pentachlorophenol and 2,3,4,6-tetrachlorophenol are extensively applied to freshly sawn lumber as antifungal agents. Curtis et al. (1972) detected up to 100 mg 2,3,4,6-tetrachlorophenol per kilogram of wood in several samples of shavings and sawdust produced from imported timber. Parr et al. (1974) surveyed fresh shavings from 32 commercial broiler houses for the presence of 2,3,4,6-tetrachlorophenol and found levels of 4 to 307 mg 2,3,4,6-tetrachlorophenol per kilogram wood shavings. Pentachlorophenol is a more widely used wood preservative than 2,3,4,6-tetrachlorophenol; however, technical grade pentachlorophenol contains 10% to 20% 2,3,4,6-tetrachlorophenol, and therefore, routine treatment of timber with pentachlorophenol as a wood preservative might include inadvertent application of 2,3,4,6-tetrachlorophenol. Also, dechlorination of pentachlorophenol yields tetrachlorophenols in wood chips and shavings. The mode of entry of anisoles into the chickens has not been established. It seems likely, however, that the anisoles, and not the parent chlorophenols, are taken up directly by the chickens. Curtis et al. (1972) conducted simulated litter experiments in which a small inoculum of tainted litter was incubated with sawdust, 2,3,4,6-tetrachlorophenol, and pentachlorophenol. Virtually quantitative conversion of 2,3,4,6-tetrachlorophenol to the anisole occurred within nine days (Figure C.5.1).

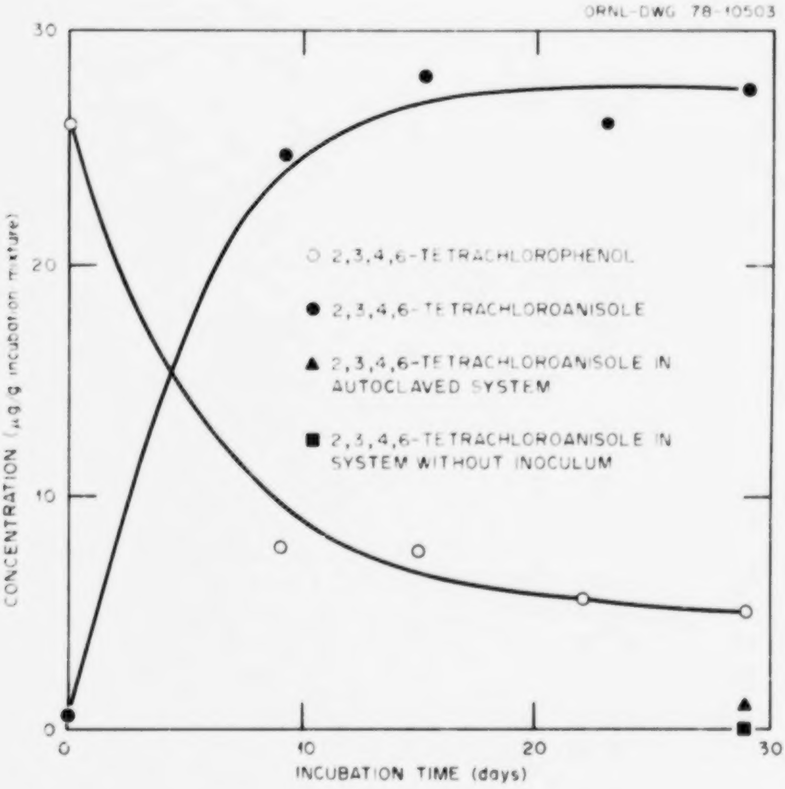

Figure C.5.1. Conversion of 2,3,4,6-tetrachlorophenol to 2,3,4,6-tetrachloroanisole in simulated broiler house litter. Source: Adapted from Curtis et al., 1972, Figure 1, p. 224. Reprinted by permission of the publisher.

The presence of 2,4,6-trichlorophenol and 2,4,6-trichloroanisole in extracts of broiler house litter has been reported by Land et al. (1975). Although these compounds were present in relatively small amounts, their contribution to the musty taint problem cannot be ignored. In experiments conducted by Land et al. (1975), pure cultures of fungi isolated from broiler house litter were capable of methylating 2,4,6-trichlorophenol to the corresponding chloroanisole. Furthermore, it was clearly demonstrated that a musty taint could be achieved in broiler chickens in the presence of 2,4,6-trichloroanisole. 2,4,6-Trichlorophenol in broiler litter may result from its presence in wood preservatives (Land et al., 1975), from the breakdown of pentachlorophenol (Land et al., 1975), or from disinfectants used in chicken houses (Orr et al., 1975).

C.5.2 BIOLOGICAL ASPECTS IN FISH AND OTHER AQUATIC ORGANISMS

C.5.2.1 Metabolism

C.5.2.1.1 Uptake and Absorption — A report by the Manufacturing Chemists Association (1972) indicated that 2,4,6-trichlorophenol may be toxic to fish; therefore, absorption of the compound by fish can be inferred. However, no information relating to uptake and absorption of higher chlorophenols was found.

C.5.2.1.2 Transport and Distribution — No information on transport and distribution of trichlorophenols and tetrachlorophenols in aquatic organisms was located.

C.5.2.1.3 Biotransformation — Data on the biotransformation of trichlorophenols and tetrachlorophenols in aquatic organisms were not available in the literature.

C.5.2.1.4 Elimination — No reports discussed the elimination of trichlorophenols and tetrachlorophenols in aquatic organisms.

C.5.2.2 Effects

C.5.2.2.1 Physiological or Biochemical Role — No evidence suggests that 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols perform any normal physiological function in aquatic organisms.

C.5.2.2.2 Toxicity

C.5.2.2.2.1 Mechanisms of action — No data dealing specifically with the mechanism of action of trichlorophenols and tetrachlorophenols in aquatic organisms were reported. The mechanism of action of these compounds in higher organisms is discussed in Section C.6.2. The applicability of this information to aquatic organisms is unknown.

C.5.2.2.2.2 General toxicity — No information on the toxicity of 2,4,5-trichlorophenol or tetrachlorophenols in aquatic organisms was found, but studies of 2,4,6-trichlorophenol toxicity were reported. The toxicity of 2,4,6-trichlorophenol to the fathead minnow (*Pimephales promelas*) has been studied (Manufacturing Chemists Association, 1972). Static bioassays determined that the 96-hr median tolerance limit of fathead minnows to 2,4,6-trichlorophenol was 0.6 mg/liter. Complete mortality of fish cultured at 2,4,6-trichlorophenol concentrations of 10 mg/liter occurred within 24 hr. Fish mortality was determined at three concentrations (1, 10, and 100 mg/liter). Because 100% mortality occurred at the two higher concentrations, extrapolation from two points was used in the calculation of the LC_{50} value. The validity of the determination is therefore questionable. The investigators also studied the toxic effects of 2,4,6-trichlorophenol on fish, microflora, and microfauna in aquaria with flow-through water systems. No significant effect on fish was seen when 2,4,6-trichlorophenol was added at a dosage of 0.06 or 0.47 mg/liter. The authors suggested that the tolerance of fish to these relatively high levels of 2,4,6-trichlorophenol indicated either a greater resistance of the fish in a more natural environment or degradation or assimilation of the compounds within the aquaria. Spectrophotometric measurement of 2,4,6-trichlorophenol content in the aquaria was performed (Table C.5.2). The 2,4,6-trichlorophenol residues determined by this method were lower than the applied dose. It is obviously difficult to draw conclusions from this type of data because measured values of 2,4,6-trichlorophenol in the static bioassays were not reported. When 2,4,6-trichlorophenol was added at an initial level of 4.15 mg/liter, all the test fish died within a few days. Because the control fish in the flow-through bioassay systems suffered a high mortality rate over the experimental period, it is difficult to evaluate these findings.

TABLE C.5.2. APPLIED VERSUS MEASURED CONCENTRATIONS OF 2,4,6-TRICHLOROPHENOL IN A FLOW-THROUGH BIOASSAY SYSTEM

2,4,6-Trichlorophenol concentration (mg/liter)	
Applied	Measured
0.06	Not detectable
0.47	0.25
4.15	1.75

Source: Adapted from Manufacturing Chemists Association, 1972, Table 16, p. 85.

Aquatic microfauna were also examined for 2,4,6-trichlorophenol in the flow-through bioassay system (Manufacturing Chemists Association, 1972). Although the instability of the control microorganisms in this study prevent a valid statistical treatment of the data, an inhibitory effect of 2,4,6-trichlorophenol on total numbers of microorganisms — particularly on the population of stalked ciliates — was indicated at 2,4,6-trichlorophenol concentrations greater than 4 mg/liter. Thus, qualitative information was gathered in these experiments, but the flow-through studies did not provide reliable quantitative data from which conclusive determinations of toxicity could be made.

Clowes (1951) reported that 2,4,6-trichlorophenol affected oxygen consumption and cell division in fertilized sea urchin eggs (*Arbacia punctulata*). Sea urchin eggs treated with 6.2 mg/liter 2,4,6-trichlorophenol consumed approximately twice as much oxygen as control eggs, and this phenomenon was accompanied by a dramatic decrease in the rate of cell division. At the limiting concentration of 6.2 mg/liter, a decreased rate of cell division was initiated in treated eggs; at a concentration of 39 mg/liter, cell division ceased entirely.

SECTION C.5

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C.6 BIOLOGICAL ASPECTS IN HUMANS

Many commonly used organochlorine compounds decompose in mammals to yield higher chlorophenols. In many cases, the only available information on the biological aspects of these chlorophenols in mammals is derived from studies dealing with this process. Although this information is indirect, it is currently the best available source. Data relating to organochlorine degradation to form higher chlorophenols are summarized in Table C.6.1. This information is useful not only in inferring some of the biological aspects of the chlorophenols, but also in indicating a possible source of exposure to higher chlorophenols for humans and other mammals.

Chemical nomenclature in studies involving organochlorine compounds must be carefully examined because common names for some of these chemicals may be confusing and misleading. For example, certain hexachlorocyclohexane isomers have been used as insecticides. Hexachlorocyclohexane may exist in four isomeric forms: alpha, beta, gamma, and delta. Lindane is the common name for γ-1,2,3,4,5,6-hexachlorocyclohexane, although lindane preparations may contain small amounts of the alpha, beta, and delta isomers. A commonly used (although incorrect) name for hexachlorocyclohexane is benzene hexachloride. Chlorobenzene compounds (which do contain an aromatic nucleus) are widely used; therefore, caution is required in reading the literature to avoid confusing benzene hexachloride (hexachlorocyclohexane) with hexachlorobenzene. In this report, hexachlorocyclohexane has been substituted whenever the original investigators used the common name benzene hexachloride.

C.6.1 METABOLISM

C.6.1.1 Uptake and Absorption

Toxicity data derived from experimental animals indicate that 2,4,5- and 2,4,6-trichlorophenol are absorbed readily from the gastroenteric tract and from parenteral sites of injection. Both trichlorophenol isomers can produce redness and edema on skin contact and, on prolonged exposure, may produce mild to moderate chemical burns of the skin. Contact with the eye induces conjunctival irritation and sometimes corneal injury and iritis (Dow Chemical Company, 1969; Gosselin et al., 1976). McCollister Lockwood, and Rowe (1961) stated, "There is no danger of poisoning from absorption (of 2,4,5-trichlorophenol) through the skin." 2,4,5-Trichlorophenol and 2,4,6-trichlorophenol apparently do not penetrate intact rabbit or guinea pig skin (Gosselin et al., 1976). No information on the rates of absorption of 2,4,5- or 2,4,6-trichlorophenol in humans or experimental animals was found.

Toxicity data indicate that 2,3,4,6-tetrachlorophenol is also readily absorbed from the gastroenteric tract and from parenteral sites of injection. Furthermore, 2,3,4,6-tetrachlorophenol dissolved in organic solvents may be absorbed directly through the skin in acutely toxic amounts (Gosselin et al., 1976). No studies of the uptake of 2,3,5,6-tetrachlorophenol in

TABLE C.6.1. CHLOROPHENOLS DETECTED IN ANIMAL TISSUE OR EXCRETION FOLLOWING ADMINISTRATION OF RELATED ORGANIC COMPOUNDS

Compound administered	Animal	Chlorophenol detected	Source
2,4,5-T (2,4,5-trichlorophenoxyacetic acid)	Sheep	2,4,5-Trichlorophenol	Clark et al., 1975
	Cow	2,4,5-Trichlorophenol	Bjerke et al., 1972
Silvex [2-(2,4,5-trichlorophenoxy)propionic acid]	Sheep	2,4,5-Trichlorophenol	Clark et al., 1975
	Cattle	2,4,5-Trichlorophenol	Clark et al., 1975
α-Hexachlorocyclohexane	Rat	2,4,6-Trichlorophenol 2,4,5-Trichlorophenol	Koransky et al., 1975
	Rat	2,4,5-Trichlorophenol 2,4,6-Trichlorophenol	Freal and Chadwick, 1973
β-Hexachlorocyclohexane	Rat	2,4,6-Trichlorophenol	Freal and Chadwick, 1973
Lindane (γ-hexachlorocyclohexane)	Rat	2,4,5-Trichlorophenol 2,4,6-Trichlorophenol 2,3,4,5-Tetrachlorophenol 2,3,4,6-Tetrachlorophenol	Chadwick and Freal, 1972
	Mouse	2,4,6-Trichlorophenol	Kurihara and Nakajima, 1974
β-Hexachlorocyclohexane	Mouse	2,4,6-Trichlorophenol 2,4,5-Trichlorophenol	Kurihara and Nakajima, 1974
Lindane (γ-hexachlorocyclohexane)	Rat	2,4,6-Trichlorophenol 2,3,4,6-Tetrachlorophenol 2,3,5,6-Tetrachlorophenol	Engst et al., 1976
δ-Hexachlorocyclohexane	Rat	2,4,5-Trichlorophenol 2,4,6-Trichlorophenol	Freal and Chadwick, 1973
4-(2,4,5-Trichlorophenoxy)butyric acid	Rat	2,4,5-Trichlorophenol	Böhme and Grunow, 1974
γ-2,3,4,5,6-Pentachlorocyclohex-1-ene	Rat	2,4,5-Trichlorophenol	Freal and Chadwick, 1973
	Rat	2,4,6-Trichlorophenol 2,3,4,6-Tetrachlorophenol 2,3,5,6-Tetrachlorophenol	Engst et al., 1976
Ronnel [O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate]	Rat	2,4,5-Trichlorophenol	Shafik, Sullivan, and Enos, 1973
Erbon [2-(2,4,5-trichlorophenoxy)ethyl 2,2-dichloropropionate]	Sheep	2,4,5-Trichlorophenol	Wright et al., 1970
Pentachlorobenzene	Rat	2,4,6-Trichlorophenol 2,3,4,5-Tetrachlorophenol 2,3,4,6-Tetrachlorophenol 2,3,5,6-Tetrachlorophenol	Engst et al., 1976

(continued)

TABLE C.6.1 (continued)

Compound administered	Animal	Chlorophenol detected	Source
1,2,4-Trichlorobenzene	Rabbit	2,4,5-Trichlorophenol	Kohli, Jones, and Safe, 1976
1,3,5-Trichlorobenzene	Rabbit	2,4,6-Trichlorophenol	Kohli, Jones, and Safe, 1976
1,2,3,4-Tetrachlorobenzene	Rabbit	2,3,4,5-Tetrachlorophenol 2,3,4,6-Tetrachlorophenol	Kohli, Jones, and Safe, 1976
1,2,3,5-Tetrachlorobenzene	Rabbit	2,3,4,5-Tetrachlorophenol 2,3,5,6-Tetrachlorophenol 2,3,4,6-Tetrachlorophenol	Kohli, Jones, and Safe, 1976
1,2,4,5-Tetrachlorobenzene	Rabbit	2,3,5,6-Tetrachlorophenol 2,3,4,5-Tetrachlorophenol	Kohli, Jones, and Safe, 1976

humans or experimental animals were located. Christensen and Luginbyhl (1975) reported that 2,3,5,6-tetrachlorophenol is lethal to mice following intraperitoneal injection. No other data relating to the rate or mechanisms of uptake of tetrachlorophenols were reported.

C.6.1.2 Transport and Distribution

Information on the transport and distribution of higher chlorophenols following their administration to experimental animals or humans is not available. These chlorophenols, however, have been detected in the tissues of animals dosed with chemically similar compounds.

Clark et al. (1975) determined the tissue distribution of 2,4,5-trichlorophenol in sheep fed 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at a level of 2000 mg/kg feed. On the basis that each animal ingested a daily ration of 3% of its body weight, this level represented 60 mg 2,4,5-T per kilogram body weight daily. The animals were maintained on a diet containing 2,4,5-T for 28 days, and tissue samples were analyzed for 2,4,5-T and 2,4,5-trichlorophenol residues at the end of this period (Table C.6.2). 2,4,5-Trichlorophenol was not detected in the fat, but the liver, kidney, and muscle contained substantial levels of 2,4,5-trichlorophenol. Withdrawal from 2,4,5-T treatment for one week before the sheep were killed resulted in a significant reduction in the residue level in muscle, but the liver and kidney tissues retained most of the 2,4,5-trichlorophenol that had accumulated. Because 2,4,5-T is probably detoxified in the liver following administration and the compound or its

TABLE C.6.2. RESIDUES OF 2,4,5-T AND 2,4,5-TRICHLOROPHENOL IN SHEEP FED 60 mg/kg 2,4,5-T DAILY FOR 28 DAYS

Tissue	2,4,5-T residue level (mg/kg body wt)		2,4,5-Trichlorophenol residue level (mg/kg body wt)	
	28-day exposure	28-day exposure plus 7-day withdrawal	28-day exposure	28-day exposure plus 7-day withdrawal
Muscle	1.00	<0.05	0.13	0.05
Fat	0.27	<0.05	<0.05	<0.05
Liver	2.29	<0.05	6.1	4.4
Kidney	27.2	0.06	0.90	0.81

Source: Adapted from Clark et al., 1975, Table IV, p. 576. Reprinted by permission of the publisher.

metabolites are excreted by the kidney, high levels of 2,4,5-trichlorophenol in these tissues are not surprising. However, it is difficult to draw conclusions from these data regarding the fate of 2,4,5-trichlorophenol administered directly.

Engst et al. (1976) detected substantial levels of higher chlorophenols in rats dosed with lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane). The rats received daily doses of 8 mg/kg body weight for 19 consecutive days. Analysis of tissues for chlorophenol residues revealed the presence of 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol in the heart. 2,3,4,6-Tetrachlorophenol and 2,3,5,6-tetrachlorophenol were detected in the liver, and the kidney contained detectable amounts of 2,4,6-trichlorophenol and the two tetrachlorophenol isomers. Quantitative residue levels were not reported, and other rat tissues were not analyzed.

Engst et al. (1976) also detected chlorophenols as metabolites in rats administered pentachlorophenol, pentachlorobenzene, and γ-2,3,4,5,6-pentachlorocyclohex-1-ene. These compounds are potential metabolites of lindane and thus may represent intermediate steps in the production of chlorophenols from lindane. Following administration of pentachlorophenol, 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol were detected in the urine. 2,4,6-Trichlorophenol and 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol were found in the urine of rats dosed with pentachlorobenzene. 2,3,4,5-Tetrachlorophenol were also present in the kidney and the blood. Metabolites of γ-2,3,4,5,6-pentachlorocyclohex-1-ene and 2,3,4,6- and 2,3,5,6-tetrachlorophenol.

C.6.1.3 Biotransformation

No studies on the metabolic fate of 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols in humans or experimental animals have been conducted, but some information on metabolic pathways can be derived from other types of experiments. Kurihara and Nakajima (1974) studied the metabolites of γ- and β-hexachlorocyclohexane following injection into mice. 2,4,6-Trichlorophenol was a major metabolite, comprising 25% of the total urinary metabolites of the two hexachlorocyclohexanes. Most of the 2,4,6-trichlorophenol excreted in the urine was present as conjugates of glucuronide and sulfate. Very little free 2,4,6-trichlorophenol was detected in the urine. Following injection of γ-hexachlorocyclohexane, 80% of the 2,4,6-trichlorophenol was excreted as sulfate conjugates, and glucuronide conjugates accounted for nearly 20% of the excreted 2,4,6-trichlorophenol. Following injection of β-hexachlorocyclohexane, sulfate and glucuronide conjugates of 2,4,6-trichlorophenol comprised 40% and 60%, respectively, of the 2,4,6-trichlorophenol excreted. Trace amounts of 2,4,5-trichlorophenol and its conjugates were also detected in the urine of mice dosed with β-hexachlorocyclohexane.

The experimental data obtained by Kurihara and Nakajima (1974) have been confirmed by Koransky et al. (1975) in experiments with rats. These investigators injected ^{14}C-labeled α-hexachlorocyclohexane intraperitoneally. The major urinary metabolites of α-hexachlorocyclohexane were

2,4,6-trichlorophenol and its conjugates. Of the total radioactivity in the urine, 3% was free 2,4,6-trichlorophenol, and nearly 40% of the total urinary activity was 2,4,6-trichlorophenol conjugates. The authors postulated that these conjugates were largely sulfate and glucuronide compounds. Small amounts of 2,4,5-trichlorophenol (<0.05% of the total urinary activity) and even smaller amounts of 2,3,4,5-tetrachlorophenol were also detected in the urine. The amounts of 2,4,5-trichlorophenol and 2,3,4,5-tetrachlorophenol present in conjugated forms were not determined.

Böhme and Grunow (1974) detected both free and conjugated 2,4,5-trichlorophenol in the urine of rats after administering the herbicide 4-(2,4,5-trichlorophenoxy)butyric acid. Free 2,4,5-trichlorophenol was also detected in the feces. Following administration of lindane to rats, Engst et al. (1976) found 2,4,6-trichlorophenol and 2,3,4,6- and 2,3,5,6-tetrachlorophenol in the urine. When pentachlorophenol was administered, 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol were detected as metabolites in the urine. To a small extent (no quantitative data were furnished), each of the four metabolites was conjugated with β-glucuronide.

Thus, evidence exists that higher chlorophenols are excreted in a conjugated form in the urine of animals. The metabolic pathway responsible for this conjugation is not known, and other metabolic pathways which may be operative in humans and other mammals following ingestion of chlorophenols or their precursors remain speculative.

C.6.1.4 Elimination

No information was found on the elimination of 2,4,5- and 2,4,6-trichlorophenol or the tetrachlorophenol isomers following direct administration of these compounds to humans or experimental animals. Data reported by Engst et al. (1976), Kurihara and Nakajima (1974), and Böhme and Grunow (1974), which were discussed in detail in Section C.6.1.3 demonstrate that chlorophenols formed as metabolites of other organochlorine compounds are frequently excreted in the urine in the conjugated form. Evidence exists (Kurihara and Nakajima, 1974) that 2,4,6-trichlorophenol formed following administration or γ- or β-1,2,3,4,5,6-hexachlorocyclohexane is excreted largely as glucuronide and/or sulfate conjugates. Other investigations have confirmed that 2,4,6-trichlorophenol is excreted primarily as a conjugate in experimental animals (Böhme and Grunow, 1974; Koransky et al., 1975). Evidence also indicates that 2,4,5-trichlorophenol and 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol are excreted as conjugates, primarily of glucuronic acid (Böhme and Grunow, 1974; Engst, 1976). It is not clear whether these compounds are excreted in experimental animals primarily as conjugates or in the free form; however, the presence of conjugates has been confirmed. Higher chlorophenols have not been detected in fecal material following administration of organochlorine compounds; therefore, the urinary route is most likely the primary route of elimination.

It is extremely difficult to extrapolate from excretion rate data taken from experiments in which the chlorophenol in question is derived from an organochlorine precursor compound. The precursor itself may be in various deposits in the body of the animal or human; thus, excretion rates will depend primarily on the lability of the precursor compound and not on any inherent characteristic of the chlorophenol. Furthermore, urinary pH interrelationships with pK_a values for different chlorophenols could be quantitatively important in urinary excretion. The data of Chadwick and Freal (1972) indicated that following a one-week exposure to lindane, rats excreted 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,5- and 2,3,4,6-tetrachlorophenol for at least one month following treatment. Shafik, Sullivan, and Enos (1973) administered ronnel [*O*,*O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate] to rats at a dosage of 234 nanomoles and found that 2,4,5-trichlorophenol produced in the animal was totally excreted in two days. The difference in excretion rates in the two studies likely depends on the chemical nature of the precursor. Clark et al. (1975) reported that after 2,4,5-T was administered to sheep, residues of 2,4,5-trichlorophenol in the liver and kidney remained relatively constant for one week after dosing was stopped. However, the 2,4,5-trichlorophenol content of the muscle dropped by a factor of 3 during the same one-week withdrawal period.

C.6.2 EFFECTS

C.6.2.1 Physiological or Biochemical Role

There is no evidence that 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenol isomers have any normal biochemical or physiological role in humans or other mammals.

C.6.2.2 Toxicity

C.6.2.2.1 Mechanism of Action — Human systemic poisonings by 2,4,5- and 2,4,6-trichlorophenol or tetrachlorophenols have not been reported. Investigations with experimental animals have indicated that 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6- and 2,3,5,6-tetrachlorophenol are capable of causing death. Lethal dosages and routes of administration for these compounds are compiled in Table A.6.4. The mechanism of toxicity has not been studied adequately. In general, trichlorophenols and tetrachlorophenols possess the ability to uncouple oxidative phosphorylation in higher animals. 2,4,6-Trichlorophenol has a convulsive action in experimental animals as well as an ability to uncouple oxidative phosphorylation. These mechanisms may explain all or part of the toxic capacity of these compounds. This question warrants further research.

According to Mitsuda, Murakami, and Kawai (1963), 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol are capable of inhibiting oxidative phosphorylation in rat liver mitochondria. 2,3,4,6-Tetrachlorophenol was the most active of the three compounds in inhibiting oxidative phosphorylation. Its activity was approximately 50% of the activity of pentachlorophenol; trichlorophenol was less active in inhibiting oxidative phosphorylation. 2,4,5-Trichlorophenol possessed approximately 30% of the activity of pentachlorophenol, and 2,4,6-trichlorophenol possessed 5% of the activity of pentachlorophenol. Farquharson, Gage, and Northover

(1958) examined the capacity of these compounds to inhibit oxidative phosphorylation in rat brain homogenate preparations and also their effects on the isolated rat diaphragm. Relatively low concentrations of each of the compounds tested (2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol) stimulated oxygen consumption in rat brain homogenates. Effective concentrations were very close to those for pentachlorophenol — a well known uncoupler of oxidative phosphorylation. Each of the higher chlorophenols mentioned, as well as pentachlorophenol, were capable of sending the isolated rat diaphragm into contracture. Trichlorophenols and tetrachlorophenols have also been reported to selectively inhibit cytochrome P-450 (Arrhenius et al., 1977).

Typical clinical signs of pentachlorophenol poisoning in experimental animals include hyperpyrexia and extremely rapid onset of rigor mortis following death. Rapid, intense onset of rigor mortis occurred within 3 to 5 min after death when pentachlorophenol, 2,3,4,6-tetrachlorophenol, and 2,4,6- and 2,4,5-trichlorophenol were injected (Farquharson, Gage, and Northover, 1958). Hyperpyrexia appeared with injection of 2,3,4,6-tetrachlorophenol in rats; 2,4,5- and 2,4,6-trichlorophenol poisoning was characterized by a slight elevation in body temperature. Although the correspondence between poisoning by pentachlorophenol and poisoning by trichlorophenols and tetrachlorophenols is not exact, there is sufficient evidence that the primary toxic mechanism of these compounds in experimental animals is inhibition of oxidative phosphorylation, thereby short-circuiting metabolism. A general property of uncouplers is the ability to inhibit the enzymes lactate dehydrogenase and hexokinase in in vitro systems. 2,4,6-Trichlorophenol and 2,3,4,6-tetrachlorophenol inhibit these enzymes in in vitro systems (Stockdale and Selwyn, 1971).

C.6.2.2.2 General Toxicity — The toxicological picture of trichlorophenols or tetrachlorophenols in experimental animals is not well documented. Because human systemic intoxication has not been reported, the toxicological picture in humans remains speculative.

Farquharson, Gage, and Northover (1958) studied the toxicology of 12 chlorophenol isomers in the rat. They separated the chlorophenols which they tested into two general categories: convulsive agents and nonconvulsive agents. Injection of phenol and the convulsive phenols, including 2,4,6-trichlorophenol, resulted in a similar syndrome. The animals experienced tremors in 40 to 120 sec following dosing, and these tremors became generalized almost immediately. The head and forepaws were sometimes affected first. When poisoning was sufficiently great for the tremors to increase in severity, intermittent convulsions were seen and the rat usually fell on its side with a loss of righting reflexes. Coma and dyspnea followed, and the rats generally lay prostrate until death. Treatment with nonconvulsive chlorophenols, including 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol, produced a slightly different toxicological picture. Hypotonia was pronounced, beginning in the hind limb within 2 to 3 min, and generally progressed to involve forelimbs and neck so that the rat was completely prostrate. Eventually, the eye reflex weakened and no withdrawal response to toe pinching was seen. There was a marked rise in temperature following injection of

2,3,4,6-tetrachlorophenol, but 2,4,6-trichlorophenol caused only a slight elevation in temperature. In all animals treated with chlorophenols, respiration was initially accelerated but then became slower as coma developed, particularly in the case of 2,3,4,6-tetrachlorophenol. 2,3,4,6-Tetrachlorophenol poisoning is unique in one other respect. Respiration ceased 0.5 to 2 min before the heart stopped beating, but with the other compounds, respiration ceased either just before or simultaneously with heart stoppage. Pronounced and rapid rigor mortis occurred with 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol. Other effects noted intermittently in poisoned animals included chromodacryorrhea, lacrimation, salivation, and diarrhea.

McCollister, Lockwood, and Rowe (1961) studied the toxicological effect of 2,4,5-trichlorophenol in rats and rabbits. In rats given acute oral doses of 2,4,5-trichlorophenol, the primary symptom was diarrhea. Rabbits were fed 20 oral doses of 2,4,5-trichlorophenol by intubation in a 28-day period. Toxicological examination showed no changes when the animals received doses of either 1 or 10 mg/kg. Very slight kidney changes were seen at 100 mg/kg, and very slight kidney and liver changes were noted at doses of 500 mg/kg. Rats were maintained on diets containing 2,4,5-trichlorophenol so that doses in the food resulted in a daily intake of 10, 30, 100, 300, or 1000 mg/kg body weight. No adverse effects were observed in the rats given 10, 30, or 100 mg/kg daily. Gross appearance and behavior, mortality, food consumption, growth, hematologic values, final average body and organ rate ratios, and gross microscopic examination of the tissues were assessed. Rats receiving 300 mg/kg or 1000 mg/kg daily sustained minor microscopic damage to the kidneys or liver. The kidneys showed a moderate degenerative change in the epithelium of the convoluted tubules and early proliferation of the interstitial tissue. Liver damage included mild centrilobular degenerative changes. Slight proliferation of the bile ducts and early portal cirrhosis were also observed. According to the pathologist who made the examinations, the changes observed in the liver and kidneys were of a mild, reversible nature and probably of minor significance.

Based on published LD_{50} values for experimental animals (Table A.6.4) and the apparent capacity of tetrachlorophenols to be absorbed directly through the skin, this compound poses the greatest hazard. Trichlorophenols appear to present relatively minor toxicological problems. According to Doedens (1963), trichlorophenols are mildly toxic. Moderate skin irritation is possible on contact, and marked irritation and possibly some corneal injury accompanies eye contact. The dusts are likely to be very irritating to the nose and throat. Tetrachlorophenols may be moderately to highly toxic when ingested and highly toxic when absorbed by the skin. Marked irritation or even a burn accompany eye contact, and dusts of these materials are also very irritating to the respiratory tract and eyes. Inhalation of dust is not recommended. One study reported a case of human systemic intoxication which involved tetrachlorophenols. Truhaut, Épée, and Boussemart (1952) described two fatal intoxications among wood treaters using a solution of pentachlorophenol and sodium tetrachlorophenate. The deaths were thought to have resulted from pentachlorophenol intoxication, although a contribution by tetrachlorophenol in the formulation cannot be excluded.

Continuous daily contact with trichlorophenols and tetrachlorophenols has reportedly caused acneform dermatitis in humans. The skin injury was caused by cutaneous contact with tetrachlorophenol or its sodium salts used for the preservation of wood. Butler (1937) described a skin condition characterized by papulofollicular lesions associated with marked hyperkeratosis and numerous comedones and sebaceous cysts in individuals handling a mixture of *o*-(2-chlorophenyl)phenol and sodium tetrachlorophenate (unknown isomer). Stingily (1940) reported on patients showing similar symptoms with certain variations; he assumed that the disturbances were caused in part by a fungus infection. These individuals were involved in the lumber industry, and cutaneous contact with sodium tetrachlorophenate was believed responsible for onset of the lesions. The lesions lasted over a period of years, and this chronicity was believed to be due to underlying fungus infections.

Kleu and Göltz (1971) discussed the case histories of ten patients suffering from chloracne due to a 15-year exposure to a trichlorophenol formulation. A psychopathological syndrome was also reported, the intensity of which increased over the 15-year period of exposure. Symptoms included decreased sexual activity, easy fatigability, irritability, muscular weakness, loss of appetite and memory, discouragement, alcohol intolerance, and loss of interest. At the time the article was written, a permanent defect was becoming evident as reduced vital psychic and intellectual capacities combined with neurasthenia and mental depression. Acute dermatitis among agricultural workers exposed to copper trichlorophenate (Karimov, 1975) and the appearance of occupational dermatitis among workers engaged in the production of 2,4,5-trichlorophenol (Zelikov and Danilov, 1974) have been reported in the Soviet Union. Bleiberg et al. (1964) reported 29 cases of acquired chloracne and 11 cases of porphyria in workers involved in the manufacture of dichlorophenol and 2,4,5-trichlorophenol. The occupational environment contained acetic acid, phenol, monochloracetic acid, and sodium hydroxide as well as 2,4-dichlorophenol and 2,4,5-trichlorophenol. It is probable that chlorodibenzo-*p*-dioxins, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, were involved in the skin and liver lesions in the above cases.

C.6.2.2.3 Carcinogenicity — Innes et al. (1969) studied the tumorigenicity of 130 organic compounds, including 2,4,6-trichlorophenol and pentachlorophenol, following continuous oral administration to mice. Pentachlorophenol showed no evidence of tumorigenicity. The 2,4,6-trichlorophenol data were ambiguous, and the authors suggested that additional evaluation is necessary.

Following a single initiating dose of dimethylbenzanthracene, repeated applications of phenol and some substituted phenols are capable of causing skin tumors in mice (Boutwell and Bosch, 1959). Mice not exposed to dimethylbenzanthracene but treated with phenol alone for long periods also showed tumor development. 2,4,5-Trichlorophenol was tested for tumorigenicity and tumor-promoting activity in a similar system (Boutwell and Bosch, 1959), and tumor-promoting activity was present; no tumor-promoting activity was seen when 2,4,6-trichlorophenol was tested in the same system. There is no evidence that 2,4,5-trichlorophenol causes tumors in the absence of an initiator (i.e., dimethylbenzantnracene). 2,4,5-Trichlorophenol was approximately the same in promoting tumor formation as phenol (Table C.6.3). Both benign and malignant tumors were formed. The hazardous effect to humans exposed to 2,4,5-trichlorophenol is not known. Prolonged skin contact is not recommended because of the potential for tumor formation and because the compound is a primary skin irritant.

TABLE C.6.3. APPEARANCE OF SKIN TUMORS IN MICE TREATED CUTANEOUSLY WITH PHENOLS FOLLOWING A CUTANEOUS DOSE OF 0.3% DIMETHYLBENZANTHRACENE IN ACETONE

Treatment[a]	Time animals examined (weeks)	Number of mice (survivors/total)	Survivors with papilloma (%)	Survivors with epithelial carcinoma (%)
Benzene control	12	12/12	0	0
10% phenol in benzene, no dimethylbenzanthracene	20	24/30	33	13
20% phenol in acetone	12	21/24	58	5
20% phenol in benzene	24	10/33	100	20
20% 2,4,6-trichlorophenol in benzene	15	26/29	0	0
21% 2,4,5-trichlorophenol in acetone	16	19/20	42	0[b]

[a] All received dimethylbenzanthracene except where stated.
[b] 5% at 30 weeks.

Source: Adapted from Boutwell and Bosch, 1959, Table 2, pp. 418-420. Reprinted by permission of the publisher.

C.6.2.2.4 Teratogenicity — Neubert and Dillmann (1972) examined 2,4,5-trichlorophenol for embryo toxicity and teratogenic effects in mice. 2,4,5-Trichlorophenol was administered daily by stomach tubes at dose rates of 0.9 mg/kg body weight or 9 mg/kg body weight on days 6 through 15 of pregnancy. No teratogenic effects (as determined by an increased number of cleft palates in offspring) were seen at either dosage level. Slightly higher embryo mortality occurred with the 9 mg/kg dosage; however, a statistical evaluation showed that the increase was only marginally significant.

C.6.2.2.5 Aplastic Anemia — One case of aplastic anemia due to pentachlorophenol and tetrachlorophenol has been reported (Roberts, 1963). The individual involved was a 21-year-old truck driver who handled lumber soaked in a product containing pentachlorophenol (3%) and tetrachlorophenol (1.5%). His clothing and hands frequently became drenched during handling of the lumber, and some oral contamination was also indicated because he was a habitual nail biter. Postmortem examination failed to reveal any other cause for the aplastic anemia, and therefore, it was concluded that the damage to the bone marrow was caused by the two chemicals. The precise contribution of the chlorophenols is unknown.

SECTION C.6

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C.7 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

C.7.1 TRENDS IN PRODUCTION AND USE

The isomers of trichlorophenol and tetrachlorophenol that are used commercially are 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol. Dow Chemical Company is the principal producer of all three chemicals, and they are marketed as Dowicide 2, Dowicide 2S, and Dowicide 6 respectively. United States production of 2,4,5-trichlorophenol in 1968 was about 13 million metric tons (Table C.7.1). Data from 1969 to 1975 reported to the U.S. International Trade Commission (U.S. International Trade Commission, 1976) were withheld. Production figures for 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol are also unavailable. Because of the lack of information in recent years, trends in production are difficult to evaluate.

TABLE C.7.1. PRODUCTION OF 2,4,5-TRICHLOROPHENOL, 2,4,5-T, AND SILVEX IN THE UNITED STATES, 1965-1975

Year	Production (metric tons)		
	2,4,5-Trichlorophenol	2,4,5-T[a]	Silvex
1965	1,817[b]	6,136	*c*
1966	2,705[b]	8,199	*c*
1967	11,465	12,344	*c*
1968	12,742	19,314	*c*
1969	*d*	5,278	725
1970	*d*	5,600	915
1971	*d*	*d*	*d*
1972	*d*	*d*	*d*
1973	*d*	*d*	*d*
1974	*d*	*d*	*d*
1975	*d*	*d*	*d*

[a] Includes 2,4,5-T acids, esters, and salts.

[b] Requirement as a 2,4,5-T intermediate is subtracted from U.S. Tariff Commission figures.

[c] Separate figure not available.

[d] Withheld to avoid disclosure.

Source: Compiled from U.S. Department of Agriculture, 1968, 1976.

The major use of 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol is direct utilization as fungicides, particularly for the preservation of wood, leather, and glue. 2,4,5-Trichlorophenol is used mainly as a fungicide and as an intermediate in the manufacture of the herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and silvex. These herbicides have wide application and are especially useful for the control of woody plants in nonagricultural areas, forests, and range lands. Silvex is also fairly effective against some aquatic weeds. Apparently, the main route by which trichlorophenols and tetrachlorophenols gain entry into the environment is by direct application and as metabolites resulting from herbicide use. A more detailed discussion of general uses is given in Section C.2.

C.7.2 SOURCES OF POLLUTION

C.7.2.1 Distribution in Air

Investigations on the sources and distribution of trichlorophenols and tetrachlorophenols in the atmosphere are not available. Because of the lack of atmospheric monitoring data for these compounds, their sources, both point and nonpoint, are only speculative. A potential for large discharges of chlorophenols into the atmosphere exists in plants where these compounds and the phenoxyalkanoic herbicides are manufactured. Volatilization of the chemicals from water, soil, foliage, and impervious surfaces may play an important role in their dispersal to the atmosphere. Incineration of containers and trash containing chlorophenols also could emit volatile products to the atmosphere.

C.7.2.2 Distribution in Aquatic Environments

Industrial waste discharge is the principal point source of water pollution. During the manufacture of chlorophenols, 2,4,5-T, and silvex, a considerable amount of chemical waste is generated by the incomplete reaction of the starting reactants, by-product formation, and incomplete recovery of desired products. These wastes contain a variety of chlorophenols and other compounds (Table B.7.2). Waste from the manufacture of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T contained amounts of 2,4,5-trichlorophenol ranging from a trace to 4.7% and of 2,4,6-trichlorophenol ranging from 2.8% to 19.5% (Table B.7.3). Other possible point sources are chemical spills and washing of containers and drums in which chlorophenols and 2,4,5-T are stored. Contamination of water may arise from (1) chlorination of phenol present either in natural water or in primary or secondary effluents of waste treatment plants (Burttschell et al., 1959; Eisenhauer, 1964; Manufacturing Chemists Association, 1972), (2) direct addition of the chemicals to waterways, (3) degradation products of silvex used for aquatic weed control, and (4) wet and dry atmospheric fallout.

Runoff from urban and agricultural watersheds could be an important nonpoint source of chlorophenols in aquatic environments. These chlorophenols are products from the degradation of 2,4,5-T and silvex, which are widely applied to control woody plants in forests and range lands and in nonagricultural areas such as irrigation and drainage ditches, roadsides, utility and railroad right-of-ways, and airfields. The contribution of runoff to the chlorophenol levels in water has not yet been documented. No other information on the distribution and sources of trichlorophenols and tetrachlorophenols in water, aquatic organisms, and sediments is available.

C.7.2.3 Distribution in Soil

Direct contamination of soils by trichlorophenols and tetrachlorophenols may be minimal. The primary source of contamination by 2,4,5-trichlorophenol could be indirect — the application of 2,4,5-T and silvex. As mentioned earlier, these herbicides are used mostly as brush killers in nonagricultural areas. The herbicides 2,4,5-T and 2,4-D were applied heavily in South Vietnam during the U.S. forest cover denial program. Estimates show that between 1961 and 1971, about 22 million kilograms of 2,4,5-T and 26 million kilograms of 2,4-D were applied on 2 million hectares of forest land in South Vietnam (Westing, 1972). Substantial sprayings in neighboring Cambodia and Laos have also been reported. Trichlorophenols and tetrachlorophenols also may be introduced indirectly to soils by the application and degradation of pentachlorophenol (Ide et al., 1972; Kuwatsuka and Igarashi, 1975) and lindane (Mel'nikov, 1974). 2,4,5-Trichlorophenol generated from herbicide applications could accumulate and move depending on its persistence and interaction with the soil.

C.7.3 ENVIRONMENTAL FATE

C.7.3.1 Mobility and Persistence in Air

The forms in which trichlorophenols and tetrachlorophenols are transported in the atmosphere are unknown. Although these chemicals can be dispersed into the atmosphere through volatilization, no investigations have been made on their presence, movement, fate, and persistence. Possibly, photodecomposition is an important mechanism of dissipation.

C.7.3.2 Mobility and Persistence in Aquatic Environments

In the aquatic environment, trichlorophenols and tetrachlorophenols may be present as the dissolved form, associated with suspended matter or bottom sediments, or absorbed by organisms. Hydrological factors such as the pattern of currents and mixing and decay and migration of organisms affect the movement of these chemicals. However, no data have been reported to support this premise.

Ingols, Gaffney, and Stevenson (1966) utilized acclimated activated sludge to study the degradation of 2,4,6-trichlorophenol. A 2,4,6-trichlorophenol concentration of as much as 100 mg/liter did not inhibit degradation (Table B.7.7). Complete ring cleavage of 2,4,6-trichlorophenol occurred in three days (Table A.7.1). The persistence of 2,4,5- and 2,4,6-trichlorophenol in aeration lagoon effluent was reported by Sidwell (1971). The initial concentrations of the compounds 2,4,5-trichlorophenol and 2,4,5-T were 18.8 and 50.0 mg/liter, respectively, and that of 2,4,6-trichlorophenol and 2,4,6-T were 18.5 and 53.0 mg/liter respectively. The pH of the acid-phenol mixture was adjusted to 7.0 just prior to mixing with the effluent. The system was maintained at 20°C to 21°C and aerated continuously throughout the experiment. The trichlorophenols disappeared rapidly; degradation was apparently complete within seven days for 2,4,5-trichlorophenol (Figure C.7.1) and within four days for 2,4,6-trichlorophenol (Figure C.7.2).

From the limited data reported above, it appears that 2,4,5- and 2,4,6-trichlorophenol are degraded rapidly in activated sludge and aeration lagoon effluent, which indicates that these media contain sufficient nutrients and large microbial populations capable of metabolizing trichlorophenols. Persistence in natural aquatic systems may vary, depending on limnological factors such as oxygen depletion, but the effect of such factors is not known.

C.7.3.3 Mobility and Persistence in Soil

The movement of trichlorophenols and tetrachlorophenols in soil largely depends on the interactions and persistence of the chemicals. More persistent compounds have a greater chance to interact with the soil, particularly by the sorption process. The extent of sorption determines whether the chemicals are carried in association with eroded soils during overland flow or are leached through the soil profile during infiltration. Soil factors affecting sorption are pH, moisture, and clay and organic matter contents.

Sorption characteristics of trichlorophenols and tetrachlorophenols have not been studied. Presumably, trichlorophenols, due to their weakly acidic properties, are sorbed by soil colloids in a manner similar to the acidic pesticides such as 2,4,5-T. Weber (1972) reviewed several investigations and concluded that acidic pesticides are sorbed only weakly by clay and organic colloids; they are considered mobile in soil and aquatic systems.

Microorganisms may play an important role in the dissipation of trichlorophenols and tetrachlorophenols in soils. The persistence of 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol was studied by Alexander and Aleem (1961) using suspensions of two silt loam soils. Participation of soil microorganisms in the dissipation of the chemicals was evidenced by a more rapid disappearance of incremental additions of the compound than of initial enrichments and by inhibition of degradation when sodium azide, a toxic agent, was added. Data showed that 2,4,6-trichlorophenol was removed rapidly from suspensions of Dunkirk and Mardin silt loams; removal time ranged from 5 to 13 days (Table A.7.2). 2,4,5-Trichlorophenol and 2,3,4,5-tetrachlorophenol persisted for more than 72 days in Dunkirk silt loams. The resistance of chlorophenols to microbial metabolism appears to be related to the positions of the chlorine atom on the aromatic nucleus. Compounds containing chlorine in the meta position, such as 2,4,5-trichlorophenol and 2,3,4,5-tetrachlorophenol, show greater persistence. Among the tetrachlorophenols, 2,3,4,6-tetrachlorophenol may degrade fastest (Ide et al., 1972). A similar relationship was also observed for the phenoxyacetic acid herbicides.

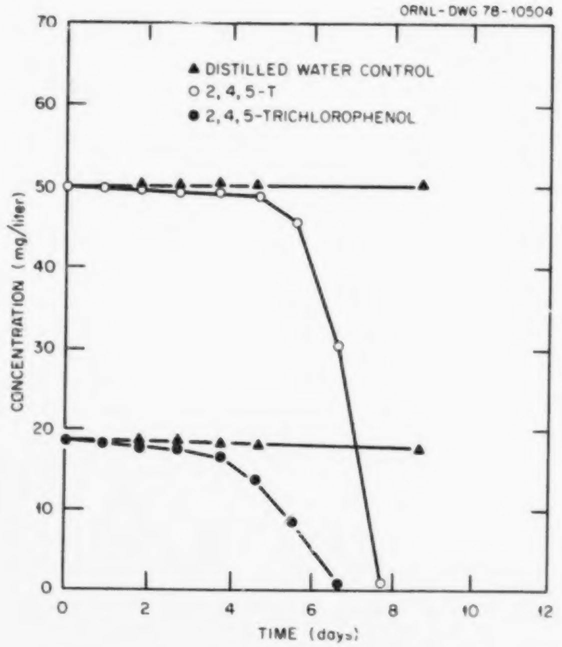

Figure C.7.1. Removal of 2,4,5-trichlorophenol and 2,4,5-T from solution in aeration basin effluent with continuous aeration. Source: Adapted from Sidwell, 1971, Figure 8, p. 70.

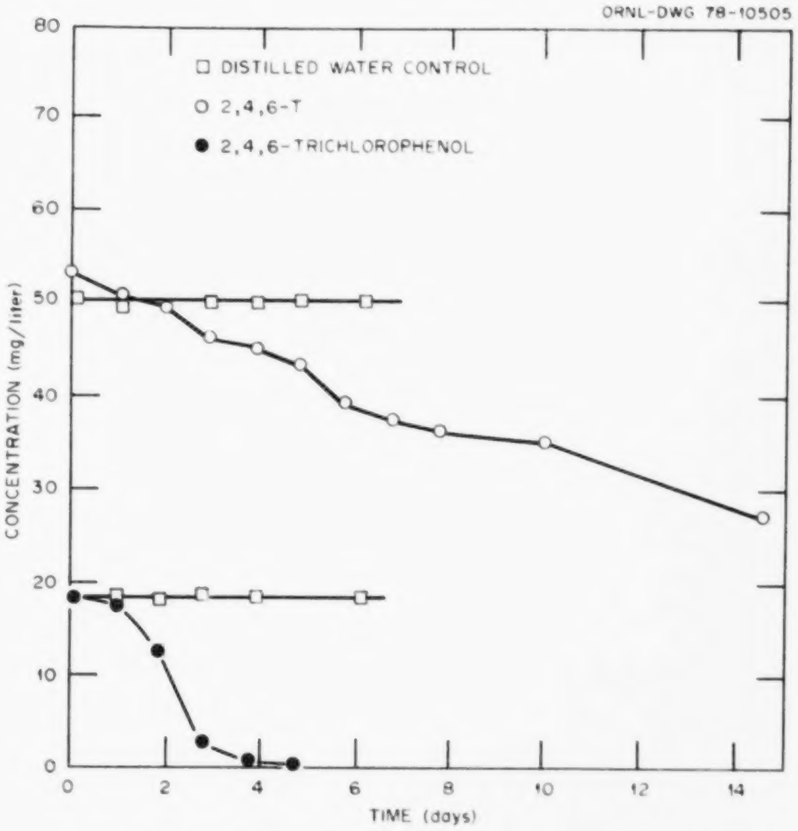

Figure C.7.2. Removal of 2,4,6-trichlorophenol and 2,4,6-T from solution in aeration basin effluent with continuous aeration. Source: Adapted from Sidwell, 1971, Figure 9, p. 71.

Ide et al. (1972) studied the persistence of the three isomers of tetrachlorophenol arising from the metabolism of pentachlorophenol in paddy soils. The isomers were incubated with soil of low organic matter content and were maintained under reducing conditions. Figure C.7.3 shows the disappearance of the isomers in four weeks; degradation was in the order 2,3,4,6-tetrachlorophenol > 2,3,5,6-tetrachlorophenol > 2,3,4,5-tetrachlorophenol. Unfortunately, degradation products were not identified. Changes in the amounts of predominant degradation products of pentachlorophenol metabolism in upland and flooded soils were investigated by Kuwatsuka and Igarashi (1975). Significant amounts of 2,3,4,5-tetrachlorophenol and 2,4,6-trichlorophenol were detected; amounts were higher under flooded conditions than in upland soils. Variable levels of 2,3,4,5-tetrachlorophenol were observed with time, but the levels of 2,4,6-trichlorophenol decreased appreciably after 20 days of incubation (Figure C.7.4). Sharpee (1973) reported the accumulation of 2,4,5-trichlorophenol during the degradation of 2,4,5-T in soils, but it did not persist as long as the herbicide.

C.7.3.4 Microbial Decomposition in Soils and Aquatic Environments

Because microorganisms seem to play a major role in the dissipation of trichlorophenols and tetrachlorophenols in the environment, these chemicals could persist longer under conditions unfavorable for microbial growth and activity. The microorganisms involved and possible mechanisms of degradation are discussed below.

The microbial degradation of trichlorophenols and tetrachlorophenols has been studied in soils (Alexander and Aleem, 1961; Ide et al., 1972), activated sludge (Ingols, Gaffney, and Stevenson, 1966; Nachtigall and Butler, 1974), lagoon effluent (Sidwell, 1971), and enrichment cultures (Chambers, Tabak, and Kabler, 1963; Chu, 1972). Nachtigall and Butler (1974) identified *Pseudomonas* sp. as the bacteria in activated sludge responsible for the metabolism of chlorophenols. Warburg respirometric studies showed that this species oxidized 2,4,6-trichlorophenol as well as monochlorophenols and dichlorophenols. Chambers, Tabak, and Kabler (1963) and Tabak, Chambers, and Kabler (1964) adapted activated sludge to phenol and found that the predominant organisms were *Pseudomonas* sp., *Achromobacter* sp., and *Flavobacterium* sp. This mixed culture was capable of metabolizing monochlorophenols, dichlorophenols, and 2,4,6-trichlorophenol. A gram-variable bacillus designated as KC-3 was isolated from a mixed bacterial culture which decreased the level of pentachlorophenol in the growth medium (Chu, 1972). This isolate utilized pentachlorophenol as a sole source of carbon for growth with concurrent mineralization of the compound to CO_2 and chloride. When 19 chlorophenols and phenol were individually tested as growth substrates, only 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol were able to support the growth of KC-3 (Table C.7.2), as indicated by substrate disappearance. Soil microorganisms capable of metabolizing trichlorophenols and tetrachlorophenols have not been reported. Possibly, the microorganisms adapted to 2,4,5-T (*Achromobacter* sp., *Mycoplana* sp., and *Streptomyces* sp.) are also active in 2,4,5-trichlorophenol metabolism (Loos, 1975). Sharpee (1973) reported the metabolism of 2,4,5-T to 2,4,5-trichlorophenol in soil and

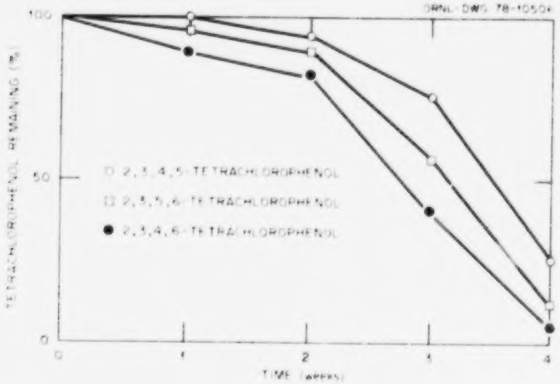

Figure C.7.3. Degradation of tetrachlorophenol isomers in paddy soil. Source: Adapted from Ide et al., 1972, Figure 7, p. 1943. Reprinted by permission of the publisher.

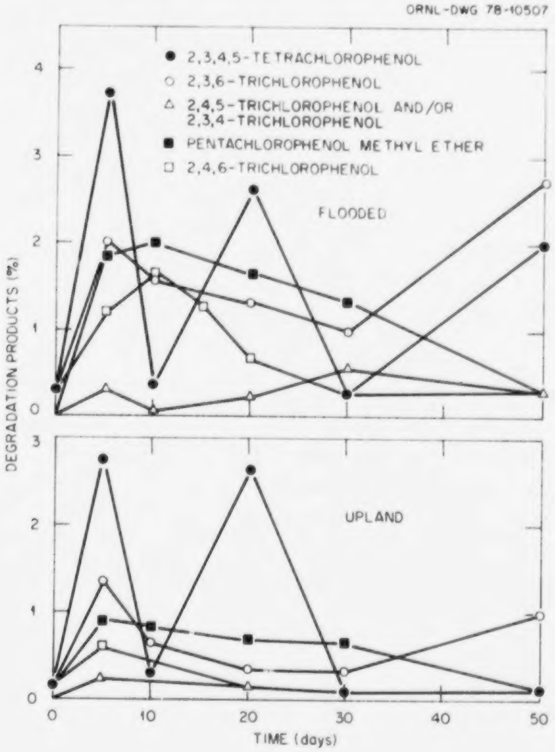

Figure C.7.4. Changes in amounts of pentachlorophenol degradation products during incubation under flooded and upland conditions. Source: Adapted from Kuwatsuka and Igarashi, 1975, Figure 4, p. 412.

TABLE C.7.2. GROWTH OF THE BACILLUS KC-3 IN CHLOROPHENOL—MINERAL SALTS MEDIA

Compound	Substrate disappearance (%)			Viable cell count (x 10^4/ml)		
	24 hr	48 hr	84 hr	0 hr	48 hr	84 hr
Pentachlorophenol	4	20	80	7.0	43	410
2,3,4,5-Tetrachlorophenol	8	71	91	7.0	270	600
2,3,5,6-Tetrachlorophenol	5	3	5	7.0	1.9	0.4
2,3,6-Trichlorophenol	3	3	2	7.0	17	9.6
2,4,6-Trichlorophenol	20	94	100	7.0	5.0	760
2,4-Dichlorophenol	0	0	0	7.0	6.4	1.7
2,6-Dichlorophenol	0	0	2	7.0	0.7	0.2
None				7.0	4.6	0.2

Source: Adapted from Chu, 1972, Table 7, p. 41. Reprinted by permission of the publisher.

aquatic systems but made no further explanation of the degradation pathways.

Fungal species such as *Aspergillus sydowi*, *Scopulariopsis brevicaulis*, and *Penicillium crustosum* which are found in wood shavings used as poultry litter have the capacity to methylate 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol to their respective anisole derivatives (Table C.7.3). *Aspergillus sydowi* was the most active in the methylation process. The anisoles resulting from biological methylation are suspected to be the main causative agents of musty taint in chickens and poultry products (Curtis et al., 1974; Land et al., 1975). Whether the methylated chlorophenols are decomposed further by the fungi is not known. Lyr (1962) also reported the enzymatic detoxification of chlorophenols, including 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol, by oxidases such as laccase, tyrosinase, and peroxidase found in wood-rotting fungi.

Chlorodioxin formation through microbial condensation of trichlorophenols and tetrachlorophenols is of interest because of the high toxicity of chlorodioxins (Helling et al., 1973). No evidence of this reaction was observed by Kearney, Woolson, and Ellington (1972) in Lakeland and Hagerstown soils incubated for 70 days with 10 to 1000 mg/liter of 2,4,5-trichlorophenol. This finding is significant because biosynthetic production of chlorodioxins from chlorophenols or herbicide metabolites is difficult to regulate under environmental conditions.

The mechanism and pathways of microbial metabolism of trichlorophenols and tetrachlorophenols have not been elucidated. As with 2,4-dichlorophenol, dechlorination, hydroxylation, and ring cleavage may be

TABLE C.7.3. METHYLATION OF 2,4,6-TRICHLOROPHENOL AND 2,3,4,6-TETRACHLOROPHENOL BY DIFFERENT FUNGAL SPECIES

Fungal species	Anisole formed (%)		
	2,4,6-Trichlorophenol, defined medium[a]	2,3,4,6-Tetrachlorophenol	
		Defined medium[a]	Simulated litter[b]
Aspergillus sydowi	77	74	100
Scopulariopsis brevicaulis	32	60	19
Penicillium crustosum	18	50	23
Mixed culture of the above three fungi	26	42	51
Sterilized control	0	0	0
Uninoculated control	0	[c]	[c]

[a] Organisms grown from six to seven days before addition of 12.4 μg 2,4,6-trichlorophenol and 17.0 μg 2,3,4,6-tetrachlorophenol in a 20-ml medium containing glucose and further incubated for five days.

[b] Organisms added to 22.5 g of sawdust containing 1220 μg 2,3,4,6-tetrachlorophenol and incubated for 29 days.

[c] No data.

Source: Compiled from Curtis et al., 1974, Table 8, p. 821; Land et al., 1975, Table 5, p. 1589. Reprinted by permission of the publishers.

involved. The cometabolism of 2,4,5-T by benzoate-grown *Brevibacterium* sp. produced 3,5-dichlorocatechol (Horvath, 1971), presumably through 2,4,5-trichlorophenol as an intermediate. The chlorocatechol was shown to be metabolized completely by the *Arthrobacter* sp. responsible for 2,4-D degradation (Bollag et al., 1968).

C.7.3.5 Photodecomposition

Many organic compounds, including chlorophenols, undergo photochemical decomposition when exposed to light. The prime requisite for such reactions is absorption of ultraviolet light. Photochemically induced degradation occurs at surfaces of soils, airborne particulates, and water. Because trichlorophenol and tetrachlorophenol reach such surfaces by various mechanisms, they are subject to exposure to sunlight and are therefore liable to photochemical degradation. Photodecomposition could be an important means of dissipation for trichlorophenols and tetrachlorophenols exposed to sunlight; however, the extent of photo-induced losses of these compounds in natural systems has not been determined.

Specific information on the photochemical reactions of trichlorophenols and tetrachlorophenols is not available. An indication of their photodecomposition can be gleaned from data obtained with closely related compounds such as pentachlorophenol and 2,4,5-T. Crosby and Hamadmad (1971) investigated the effect of ultraviolet light on pentachlorophenol in methanol and aqueous suspension. 2,3,5,6-Tetrachlorophenol was the only product detected in methanol solution. When an aqueous suspension of pentachlorophenol was irradiated, only small amounts of 2,3,5,6-tetrachlorophenol were detected; the major product was humic acid.

Photodecomposition of the herbicide 2,4,5-T in aqueous solution by ultraviolet light yielded products analogous to those produced by irradiation of 2,4-D (Figure C.7.5). The major products were 2,4,5-trichlorophenol and 2-hydroxy-4,5-dichlorophenoxyacetic acid; colored polymeric products were eventually formed. Photolysis involved cleavage of the ether bond and replacement of the ring chlorine by hydrogen and hydroxyl groups. The rate of photolysis, although more rapid at pH 8 than at pH 3, was slow compared with the rates for 4-chlorophenoxyacetic acid and 2,4-D. However, under the same conditions, the photolysis rate of 2,4,5-trichlorophenol was rapid, indicating that side chain oxidation was the rate-limiting step in 2,4,5-T photodecomposition.

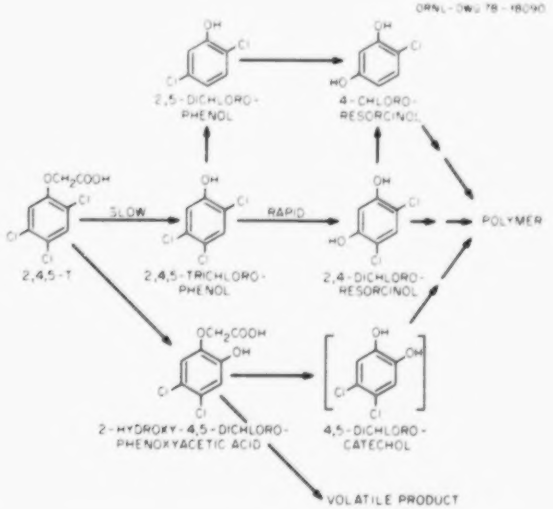
ORNL-DWG 78-18090
OH
Cl
Cl
2,5-DICHLOROPHENOL
OH
Cl
HO
4-CHLORORESORCINOL
OCH2COOH
Cl
Cl
Cl
2,4,5-T
SLOW
OH
Cl
Cl
Cl
2,4,5-TRICHLOROPHENOL
RAPID
OH
Cl
HO
Cl
2,4-DICHLORORESORCINOL
POLYMER
OCH2COOH
OH
Cl
Cl
2-HYDROXY-4,5-DICHLOROPHENOXYACETIC ACID
OH
OH
Cl
Cl
4,5-DICHLOROCATECHOL
VOLATILE PRODUCT

Figure C.7.5. Proposed photodecomposition pathway for 2,4,5-T in aqueous solution. Source: Adapted from Crosby and Wong, 1973, Figure 2, p. 1053. Reprinted by permission of the publisher.

The possibility of chlorodioxin formation during photolysis of environmental 2,4,5-trichlorophenol is of concern, although no data have been reported. Crosby and Wong (1973) detected no chlorodioxin production during the photolysis of 2,4,5-T.

C.7.3.6 Pyrolysis

It has been reported that commercially produced higher chlorophenols and 2,4,5-T are contaminated with chlorodioxins, compounds which have teratogenic effects and high toxicity (Firestone et al., 1972; Woolson, Thomas, and Ensor, 1972). Dioxins are formed when reaction temperatures for producing chlorophenols exceed 160°C under pressure. Concern over environmental contamination by chlorodioxins stems from the widespread application of 2,4,5-T (Helling et al., 1973). Chlorodioxins could also be introduced into the environment by incineration of solid waste containing chlorophenols and chlorophenol-treated materials or by heating fat which is contaminated with chlorophenols. Pyrolytic condensation of chlorophenols to chlorodioxins has been demonstrated (Higginbotham et al., 1968; Langer, Brady, and Briggs, 1973). Langer, Brady, and Briggs (1973) reported chlorodioxin yields of 80%, 30%, 15%, <1%, and <3% for the sodium salts of pentachlorophenol, 2,3,4,6-tetrachlorophenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, and 2,4-dichlorophenol respectively. Data suggest that a high degree of chlorination and chlorine in the ortho position on the aromatic ring, combined with thermal treatment, favor dioxin formation. The pyrolytic method of converting chlorophenols to dioxins was used by Flick, Firestone, and Higginbotham (1972) and Higginbotham et al. (1968) to obtain samples for toxicological evaluation.

Results of laboratory studies involving the heating of concentrated phenols at controlled temperatures do not necessarily duplicate conditions encountered in the burning of waste materials. Temperatures encountered in incineration are higher, and chlorophenols may vaporize before chlorodioxins can be formed. For example, Stehl et al. (1973) showed that octachlorodibenzo-*p*-dioxin levels did not increase when wood or paper treated with pentachlorophenol was combusted. More recently, Ahling and Johansson (1977) found that when pentachlorophenol was combusted at 600°C or 800°C, octachlorodioxin formation did not occur.

C.7.4 WASTE MANAGEMENT

Chlorophenols from various sources and/or reactions are found in natural waters and wastewater. Concentrations range from low in natural waters to high in wastewater effluents coming from the manufacture of chlorophenols and phenoxyalkanoic herbicides. Treatment processes applicable to wastewaters containing trichlorophenol and tetrachlorophenol and the removal of these chemicals from drinking waters are discussed in this section.

C.7.4.1 Primary Treatment

Primary treatment consists essentially of settling solids after screening off the larger materials. No reports on the effectiveness of settling as a means of removal of trichlorophenols and tetrachlorophenols from wastewater are available.

C.7.4.2 Secondary Treatment

Secondary treatment uses biological processes for the removal of organic matter from wastewater. Biological methods used in waste treatment include trickling filters, activated sludge, and oxidation ponds. These methods are based on the principle that microorganisms utilize organic materials in wastewaters as a source of energy and in so doing convert them to simpler, less toxic substances. Limited evidence indicates that trichlorophenols and tetrachlorophenols are biodegradable; consequently, secondary treatment should provide an excellent method for the removal of these chemicals. Sidwell (1971) investigated biological treatment of wastes generated from the manufacture of 2,4-D and 2,4,5-T by using a combined aerated lagoon and stabilization pond. These experiments are described in Section B.7.4.2. In vitro experiments with individual chlorophenols, including 2,4,5- and 2,4,6-trichlorophenol, and corresponding chlorophenoxy acids diluted with aeration lagoon effluent indicated that these compounds are decomposed in less than seven days (Figures C.7.1 and C.7.2).

C.7.4.3 Soil Disposal

Soil disposal can be a means of biological treatment of chlorophenolic wastes. However, the possibility of treating wastes which contain trichlorophenols and tetrachlorophenols by this method has not been explored.

C.7.4.4 Chemical Oxidation

Chlorine, besides being used to disinfect drinking water and effluents from primary and secondary treatment plants, is also utilized for the oxidation of chlorophenols and other organic compounds. According to Burttschell et al. (1959) and confirmed by others (Eisenhauer, 1964; Manufacturing Chemists Association, 1972), the chlorination of phenol proceeds with stepwise substitution in the 2, 4, and 6 positions of the aromatic ring (Figure C.7.6). The ultimate chlorophenol product is 2,4,6-trichlorophenol, which after further chlorination is oxidized to form a mixture of nonphenolic products. The rate of reaction of aqueous chlorine and phenol to form chlorophenols is highly pH dependent, with the maximum rate occurring in the neutral or slightly alkaline pH range (pH 7 to 8) (Lee and Morris, 1962).

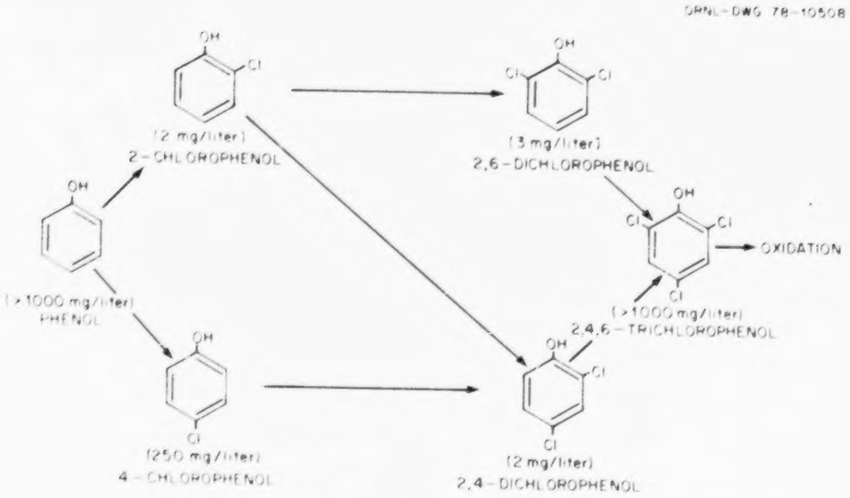
ORNL-DWG 78-10508
OH
Cl
(2 mg/liter)
2-CHLOROPHENOL
OH
Cl
Cl
(3 mg/liter)
2,6-DICHLOROPHENOL
OH
(>1000 mg/liter)
PHENOL
OH
Cl
Cl
Cl
OXIDATION
(>1000 mg/liter)
2,4,6-TRICHLOROPHENOL
OH
Cl
(250 mg/liter)
4-CHLOROPHENOL
OH
Cl
Cl
(2 mg/liter)
2,4-DICHLOROPHENOL

Figure C.7.6. Reaction scheme for the chlorination of phenol. Numbers in parentheses indicate odor threshold concentrations. Source: Adapted from Burttschell et al., 1959, Figure 3, p. 212. Reprinted with permission of the American Water Works Association from JOURNAL AWWA, Volume 51, copyrighted 1959.

C.7.4.5 Ion Exchange

Although ion-exchange resins may have the capacity to absorb trichlorophenols and tetrachlorophenols, this ability has not been demonstrated.

C.7.4.6 Activated Carbon Adsorption

Activated carbon is effective in adsorbing several organic compounds from water. Investigations specifically dealing with the utilization of activated carbon for the removal of trichlorophenols and tetrachlorophenols have not been performed. However, the adsorption of various phenoxyacetic acids by charcoal may provide insight into the possible adsorption behavior of the various trichlorophenol and tetrachlorophenol isomers. Leopold, van Schaik, and Neal (1960) studied the removal of 18 phenoxyacetic acids from water by activated carbon to show the relationship between molecular structure of chlorinated phenoxyacetic acids and adsorption on activated carbon (Table C.7.4). One, two, and three chlorine substitutions in the phenyl ring increased the adsorptive properties of the molecule. An inverse correlation between adsorption and solubility was found; successive chlorinations decreased solubility and increased adsorption. Apparently, trichlorophenols and tetrachlorophenols, due to their extremely low water solubilities, also are adsorbed by charcoal quite extensively.

TABLE C.7.4. ADSORPTION OF CHLORINATED PHENOXYACETIC ACIDS BY ACTIVATED CARBON

Compound	Adsorption[a] (%)	Solubility (x 10^{-3} *M*)
Phenoxyacetic acid	8	110
2-Chlorophenoxyacetic acid	29	6.85
3-Chlorophenoxyacetic acid	35	12.65
4-Chlorophenoxyacetic acid	35	5.13
2,3-Dichlorophenoxyacetic acid	58	1.55
2,4-Dichlorophenoxyacetic acid	49	2.36
2,5-Dichlorophenoxyacetic acid	48	2.42
2,6-Dichlorophenoxyacetic acid	32	7.05
3,4-Dichlorophenoxyacetic acid	46	2.07
3,5-Dichlorophenoxyacetic acid	53	4.35
2,3,4-Trichlorophenoxyacetic acid	64	0.80
2,3,5-Trichlorophenoxyacetic acid	64	1.00
2,3,6-Trichlorophenoxyacetic acid	43	2.40
2,4,5-Trichlorophenoxyacetic acid	65	1.05
2,4,6-Trichlorophenoxyacetic acid	51	0.97
3,4,5-Trichlorophenoxyacetic acid	70	1.15
2,3,4,6-Tetrachlorophenoxyacetic acid	59	0.39
2,3,4,5,6-Pentachlorophenoxyacetic acid	66	0.18

[a] Equimolar concentrations, 10^{-4} *M*.

Source: Adapted from Leopold, van Schaik, and Neal, Tables 1 and 2, p. 51. Reprinted by permission of the publisher.

SECTION C.7

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C.8 ENVIRONMENTAL INTERACTIONS AND THEIR CONSEQUENCES

C.8.1 ENVIRONMENTAL CYCLING OF TRICHLOROPHENOLS AND TETRACHLOROPHENOLS

The sources, distribution, and fate of 2,4,5- and 2,4,6-trichlorophenol (mention of trichlorophenols in the ensuing discussion includes both of these isomers and excludes other trichlorophenol isomers) and tetrachlorophenols in soils, aquatic environments, and the atmosphere are discussed in Section C.7. Figure A.8.1 depicts the possible cycling of these chemicals in the environment and indicates the probable sources of contamination, interactions, and sinks. This scheme should be interpreted with caution because information gaps make it difficult to ascertain patterns of distribution, accumulation, and flow of the chemicals into various parts of the environment.

Contamination of soil and aquatic systems could occur through direct application of the chemicals or compounds derived from them, discharge of industrial waste and sewage, or chlorination of water containing phenol and/or lower chlorophenols. The relative contribution from each source has not been determined. However, contamination probably results mainly from direct usage of 2,4,5- and 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol as fungicides for wood preservation and from application of pesticides such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), silvex, ronnel, lindane, and pentachlorophenol, which upon degradation yield one or more of the trichlorophenols and tetrachlorophenols as metabolite(s). Furthermore, technical pentachlorophenol contains 10% to 20% 2,3,4,6-tetrachlorophenol as a contaminant (Parr et al., 1974). Significant contamination may be contributed by discharge of chlorophenolic wastes from chemical plants producing chlorophenols and related products. Although trichlorophenols and tetrachlorophenols are quite susceptible to volatilization, their presence in the atmosphere and their subsequent redeposition during precipitation and fallout has not been documented. Volatilization and leaching from treated material, particularly wood, can occur to some extent.

Trichlorophenols and tetrachlorophenols may undergo physicochemical and biological interactions with various components of the environment. The extent of sorption by soil colloids determines whether the chemicals are carried in association with eroded soil during overland flow or are leached through the soil profile during infiltration. Unfortunately, data on sorption of these chemicals by soil and by sediments and airborne particulate materials are not available. They are probably sorbed extensively by natural sorbents due to their weakly acidic nature and low water solubility, although binding might be relatively weak, making them moderately mobile. The rates of chemical and biological transformations determine their persistence and subsequent degree of movement and transport in a particular segment of the environment. Chemical oxidation can occur during treatment of wastewater (Section C.7.4.4), and photodecomposition is a possibility when these chemicals are exposed to sunlight (Section C.7.3.5). Microorganisms may play a major role in the dissipation of trichlorophenols and tetrachlorophenols (Section C.7.3). Possibly, microbial degradation is the main mechanism by which these chemicals are removed from the environment. The degree of metabolism and mode of distribution of absorbed or ingested trichlorophenols and tetrachlorophenols in plants, wildlife, domestic animals, and other mammals can influence biological accumulation. If these chemicals are bioaccumulated and persist, they can be returned to the soil and water systems during decay and excretion or be consumed by higher biota, including humans, through the food chain. There is a lack of information regarding the metabolic fate and distribution of trichlorophenols and tetrachlorophenols in higher organisms.

C.8.2 TRICHLOROPHENOLS AND TETRACHLOROPHENOLS IN FOOD

Agricultural crops and domestic animals may indirectly become contaminated with trichlorophenols and tetrachlorophenols by absorption and/or ingestion of the pesticides 2,4,5-T, silvex, lindane, and pentachlorophenol. Kohli, Weisgerber, and Klein (1976) found that lindane absorbed from nutrient solution by lettuce plants was metabolized to free and conjugated 2,3,4,6-tetrachlorophenol. However, no reports indicate the presence and metabolic fate of trichlorophenols and tetrachlorophenols in field-grown crops. Residues of 2,4,5-trichlorophenol occurred in the kidney and liver of sheep and cattle fed with high concentrations (300 to 2000 mg/kg) of 2,4,5-T and silvex (Clark et al., 1975). Negligible residues were found in fat and muscle. 2,4,5-Trichlorophenol has been found in milk and cream of cows fed diets containing 100 to 1000 mg/kg 2,4,5-T (Bjerke et al., 1972). Milk and cream of cows that received 10 to 30 mg/kg of 2,4,5-T were free from detectable amounts of 2,4,5-trichlorophenol. Large amounts of 2,4,5-T and silvex, such as the concentrations used in the above studies, are not likely to be found in feed, forage, and pasture. Proper use of these herbicides should result in negligible residues of 2,4,5-trichlorophenol in meat and milk.

Because of the extensive use of wood shavings as a cheap source of litter, largely for broiler chickens, but also for turkeys, ducks, pigs, and cattle, continued use of shavings originating from preservative-treated wood may constitute an environmental hazard. Furthermore, spent litter is being used increasingly as a constituent of animal feeds. Limited information indicates that contamination of foodstuff by residues of trichlorophenols and tetrachlorophenols appears unlikely. However, the occurrence of musty taint in chickens due to the presence of 2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole reduces appreciably the quality of poultry meat and eggs (Curtis et al., 1974; Land et al., 1975). These anisoles are formed through fungal methylation of 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol present in wood shavings used as broiler litter. Substantial amounts of 2,3,4,6-tetrachlorophenol, ranging from 4 to 307 mg/kg, have been detected in wood shavings and sawdust originating from preservative-treated wood (Curtis et al., 1972; Parr et al., 1974). These figures imply that a large volume of 2,3,4,6-tetrachlorophenol is used as a wood preservative, but the contribution of 2,3,4,6-tetrachlorophenol from pentachlorophenol, either as a contaminant or degradation product, should not be discounted. The presence of 2,4,6-trichlorophenol in broiler litter may result either from its use as a wood preservative (Land et al., 1975), from degradation of pentachlorophenol (Land et al., 1975), or from disinfectants used in chicken houses (Orr et al., 1975).

Potential hazards exist probably not through contamination of foodstuff but in the use of wood shavings for litter for domestic animals and introduction of chlorodioxins into the environment via pyrolysis. More investigations are needed in order to appraise properly the environmental hazards associated with the use of trichlorophenols and tetrachlorophenols.

SECTION C.8

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PART D

PENTACHLOROPHENOL

D.1 SUMMARY

D.1.1 DISCUSSION OF FINDINGS

Pentachlorophenol is a white, needlelike, crystalline solid. Because of its high degree of insolubility in water, the water-soluble sodium salt is often substituted for many uses. Although pentachlorophenol is only slightly soluble in water, it is readily soluble in organic solvents and is sufficiently volatile to distill with steam. Its vapor pressure is relatively low, but losses of pentachlorophenol from soils, water, and preservative-treated items through volatilization may occur. The compound is relatively stable and is rather inert chemically. Although it is not subject to easy oxidative coupling or electrophilic substitution reactions common to most phenols, the polar hydroxyl group tends to facilitate biological degradation. Any monovalent alkali metal salt of pentachlorophenol is soluble in water, but the protonated (phenolic) form is virtually insoluble. Hence, transport of pentachlorophenol in water depends largely on the pH of the system. Pentachlorophenol and its salts are extensively used as broad-spectrum biocides. About 90% to 95% of the amount produced in the United States is used in the preservation of wood; the remaining 5% to 10% has a large number of applications in industry and agriculture. The compounds have been used as herbicides or preharvest desiccants, as algicides, as molluscicides for eradication of a snail which serves as intermediate host for the human schistosomes, and in food processing plants and pulp mills for control of slime and mold. Pentachlorophenol and sodium pentachlorophenate also have been used as fungicides and/or bactericides in the processing of cellulosic products, starches, adhesives, paints, leathers, oils, rubber, textiles, and wooden crates used for packaging raw agricultural products. Approximately 25,000,000 kg of pentachlorophenol and sodium pentachlorophenate are produced annually, and because of the wide distribution, a potential for environmental contamination exists.

A variety of qualitative and quantitative techniques have been developed for the analysis of pentachlorophenol. In general, colorimetric and oxidation methods of analysis are less sensitive and specific than chromatographic methods. The most widely used and effective technique for analysis of pentachlorophenol in many sample types is gas-liquid chromatography. The electron-capture detector is used routinely because of its high sensitivity for quantities in the nanogram to picogram range. When rigorous identification is required, final confirmation may be made by ultraviolet or infrared spectrophotometry. Efficient extraction of pentachlorophenol from soils, water, and biological materials depends on many factors determined by the type of sample to be analyzed. The advantages and disadvantages of different methods of sample storage, preparation, and analysis are discussed in Sections D.2.6.1 and D.2.6.2.

Pentachlorophenol is extremely toxic to almost all forms of bacteria, algae, and fungi. For this reason, pentachlorophenol is a well-known and widely used antimicrobial substance. Detailed information on the mechanism of pentachlorophenol toxicity to microorganisms is scant. It is believed that uncoupling oxidative phosphorylation is of major importance in the toxicity of pentachlorophenol to bacteria. However, other vital bacterial processes must also be affected because anaerobes (bacteria not dependent on oxidative phosphorylation for their energy source) are also sensitive to this compound. An effect of pentachlorophenol on the generation of ATP by substrate-level phosphorylation has been reported. Thus, the compound not only affects oxidative phosphorylation, but also affects either the generation of ATP at the level of glycolysis or the effective utilization of the ATP generated. Bacteria resistant to the toxic effect of pentachlorophenol and some strains capable of degrading pentachlorophenol have been isolated. In most cases, both resistant strains and those able to degrade pentachlorophenol were isolated from sites exposed routinely to the compound (e.g., pentachlorophenol-treated wood and soil from the grounds of wood treatment plants). The prevalence of pentachlorophenol-resistant bacteria in the general environment is unknown. The high toxicity of pentachlorophenol to most fungi determines its effectiveness as a wood preservative. Some fungal species have been isolated which are capable of tolerating pentachlorophenol and in some cases detoxifying it. The mechanism of pentachlorophenol detoxification by fungi is not well understood; however, methylation of the compound to produce pentachloroanisole is a common mechanism. Pentachlorophenol is also highly toxic to algae and has been used as an algicide in paddy fields and industrial facilities. Algae tolerant to the effect of pentachlorophenol have not been described, and metabolism of pentachlorophenol by algae has not been reported.

Pentachlorophenol is absorbed rapidly by the roots of sugar cane, but it is not readily translocated, remaining primarily at the site of treatment until metabolized or released from the plant tissue. Some translocation has been demonstrated in cotton plants following foliar application. It is not known whether pentachlorophenol readily translocates in other plants, and the subject warrants further investigation.

Pentachlorophenol is highly toxic to plants and has been used as both a contact and preemergence herbicide. Uncoupling of oxidative phosphorylation likely is responsible for the phytotoxic effects. Germination suppression may be caused by phytate decomposition in germinating seedlings. Severe damage to tomato plants and conifer seedlings planted in pentachlorophenol-treated wooden flats has been reported. In at least one case, the phytotoxicity was ascribed to volatilization of pentachlorophenol from the wood and subsequent atmospheric transport. Severe damage was reported in high-density apple orchards where posts freshly treated with pentachlorophenol were used as supports for the trees. Except for herbicide use, exposure of plants to pentachlorophenol should be strictly avoided. Pentachlorophenol is also toxic to aquatic plants; 50% lethality of duck weed (*Lemna minor*) occurs at a concentration of 0.32 mg/liter. Furthermore, pentachlorophenol reportedly increases the number of chromosomal abnormalities in root tip preparations of water hyacinth and causes abnormal mitoses in the European broad bean.

Pentachlorophenol is toxic to wild and domestic animals following cutaneous absorption or ingestion. No information on the possible respiratory toxicity of pentachlorophenol or sodium pentachlorophenate to wild and domestic animals is available. Pentachlorophenol is absorbed readily by the cutaneous route during direct, long-term exposure to freshly treated wood. Losses of sows and their litters have been reported when pigs have been confined to farrowing houses freshly, or inappropriately (i.e., excessive quantities), treated with pentachlorophenol in the absence of bedding. This effect can be reversed by appropriate treatment and provision of bedding to prevent direct contact. Swine, sheep, and cattle have been exposed to pentachlorophenol-treated gates, watering troughs, feeding troughs, salt boxes, posts, electric fence stakes, and corral timbers for up to two months. No apparent detrimental effects on the animals were noted; however, the pentachlorophenol levels in the animals were not determined, and the experiments were insufficient to evaluate chronic long-term effects of pentachlorophenol-treated wood on livestock. Although the lack of information prevents definitive conclusions, preservative-treated wood likely poses no serious hazard to domestic animals if the wood is properly treated and used.

Pentachlorophenol is apparently repellent to animals. Cattle avoid pasture treated with a herbicidal formulation of pentachlorophenol, and rats decrease their food intake when their diet includes pentachlorophenol at levels of 1000 ppm or more. Cats refuse diets containing 125 ppm. Nevertheless, domestic animals have ingested sufficient amounts of pentachlorophenol to cause death. Substantial quantities of pentachlorophenol (120 to 140 mg/kg body weight) administered in the form of pentachlorophenol-impregnated sawdust were required to cause death in calves and sheep. Based on a standard loading approximating pentachlorophenol levels in the outer layer of treated wood, ingestion of approximately 600 g of pentachlorophenol-treated wood would be required to cause acute lethal intoxication. Therefore, it seems unlikely that acute toxicity will result from ingestion of treated wood over a short period. The question of chronic toxicity from pentachlorophenol-treated wood remains an open one. Despite the repellent characteristics of pentachlorophenol, the hazard posed by concentrated solutions of the compounds in the vicinity of domestic animals is quite real. Poisoning resulting from the consumption of a solution of pentachlorophenol in kerosene by a thirsty cow has been reported. The lesson is clear — concentrated solutions of these substances should be removed from the vicinity of domestic animals. However, the hazard posed by pentachlorophenol-treated wood is not clear-cut.

Whether pentachlorophenol is absorbed by the cutaneous, respiratory, or gastrointestinal route, the compound is transported by the blood. High concentrations have been detected in the kidneys and livers of animals dosed with the compound. Based on investigations with experimental animals, biotransformation in domestic and wild animals probably involves the formation of pentachlorophenol conjugates (including glucuronides), tetrachlorohydroquinone, tetrachlorohydroquinone conjugates, and chloranil. Metabolism probably occurs primarily in the liver. Pentachlorophenol is excreted in the urine and feces of animals dosed with the compound. The kinetics of excretion in domestic animals have been partially determined.

The toxic effect of pentachlorophenol probably lies in its ability to uncouple oxidative phosphorylation, thereby short-circuiting the energy metabolism of the animal. Serious skin damage and necrosis accompany cutaneous exposure to either pentachlorophenol in petroleum solvents or sodium pentachlorophenate powder. Conversely, pentachlorophenol powder causes only slight skin redness in the rabbit. Cutaneous exposure of domestic animals to pentachlorophenol should be avoided; skin damage occurs as well as absorption through the skin which may result in systemic toxicity. Symptoms of severe systemic pentachlorophenol poisoning include hyperpyrexia, depression, rapid respiration, weakness, death without struggle, and rapid onset of rigor mortis following death. Autopsy results of fatally poisoned animals have indicated pathological changes of the kidney, liver, spleen, stomach, intestinal tract, respiratory tract, and urinary bladder.

Extensive loss of wildlife, including the death of fish, frogs, snails, and birds, has been reported following the widespread application of sodium pentachlorophenate as a molluscicide in Surinam, South America. The lethal effect of pentachlorophenol on snail kites (a species of bird which feeds almost exclusively on snails) is especially noteworthy because these animals were exposed by consuming pentachlorophenol-contaminated snails. Substantial levels of pentachlorophenol were also noted in birds which were not killed by the compound, indicating that contamination of wildlife in the area was fairly widespread. The effect of the sublethal pentachlorophenol levels found in wildlife was not determined. However, the use of pentachlorophenol as a molluscicide on such a large scale is hazardous to wildlife. Less toxic, more selective pesticides should be found.

Long-term chronic effects in wild and domestic animals following exposure to small quantities of pentachlorophenol have not been documented. Some experiments have determined the effects of moderate pentachlorophenol intake by domestic and experimental animals over medium lengths of time. Pentachlorophenol was administered to sheep and calves in the form of impregnated sawdust to simulate the ingestion of pentachlorophenol-treated wood by farm animals. Noticeable systemic effects, including loss of body weight and decline in general condition, were noted in sheep that were daily force-fed pentachlorophenol at levels of 30 mg/kg body weight for 10 to 20 days. To achieve these levels of pentachlorophenol intake, animals were force-fed with 30 g of sawdust per day. Similar systemic effects were seen in calves fed 35 mg pentachlorophenol per kilogram body weight daily for 10 to 20 days, which corresponded to an intake of 170 g sawdust per day. In these experiments, systemic effects were only noticeable at daily intake levels above 30 mg pentachlorophenol per kilogram body weight. The question of whether lower intakes of pentachlorophenol over longer periods may cause more subtle effects in domestic animals has not been answered. Rats fed diets containing pentachlorophenol at levels between 50 and 200 mg/kg food had increased liver weights with concomitant increases in microsomal enzyme activity, accompanied by lower hemoglobin and hematocrit values. Furthermore, a dose-related decrease in kidney calcium deposits occurred. The significance of these changes to the physiology of rats is unknown. Although there is obvious danger of acute systemic toxicity following absorption of large quantities of pentachlorophenol, little evidence suggests that other more subtle hazards are associated with uptake of low or chronic amounts.

Fish and aquatic organisms remove pentachlorophenol and/or sodium pentachlorophenate readily from surrounding water, but the route of uptake is unclear. Following uptake, high pentachlorophenol levels have been detected in the gills, kidneys, hepatopancreas, and gallbladder. Very high levels in the gallbladder are particularly noteworthy. It has been suggested that pentachlorophenol is detoxified following transfer to the hepatopancreas and is transported partly to the gallbladder and the bile. Many aquatic organisms, such as the short-necked clam and goldfish, readily metabolize pentachlorophenol by conjugation to sulfate and excrete pentachlorophenol in a conjugated form. Following absorption of pentachlorophenol by goldfish, initial elimination appears to be rapid; approximately 80% of the pentachlorophenol taken in by the fish is excreted within 40 hr. However, 20% of the compound remains in the fish for longer than 60 hr, and its fate is unknown. In the absence of a storage mechanism in the fish, the compound is presumably eliminated over a relatively long period. Some investigators have suggested that the slow excretion kinetics for goldfish may result from storage of pentachlorophenol in the gallbladder or perhaps in the lipids.

Pentachlorophenol is toxic to fish and other aquatic organisms when large amounts reach the receiving water. Numerous fish kills have been reported from seepage of pentachlorophenol-contaminated effluents or from spillage of the concentrated compound into waterways. Herbicidal and molluscicidal uses of the compound have been hazardous to fish and invertebrate populations. Aquatic species vary in their susceptibility to pentachlorophenol toxicity. Some fish species tolerate pentachlorophenol levels as high as 600 μg/liter, but more sensitive species perish at concentrations as low as 30 μg/liter. Therefore, substantial amounts should not be allowed to reach waterways. The embryonic development of fish and other aquatic organisms is affected at pentachlorophenol concentrations as low as 50 μg/liter. More subtle chronic effects may occur in aquatic communities exposed to pentachlorophenol residues. Effects on the growth and food conversion efficiency of the sockeye salmon have been reported at pentachlorophenol levels as low as 2 μg/liter. Eels exposed to pentachlorophenol and then placed in a fresh, pentachlorophenol-free medium showed changes in a number of blood parameters and alterations in liver enzyme activity which persisted despite the fish spending two months in "clean" water. It is not clear whether permanent damage to enzyme systems occurred or whether low pentachlorophenol levels in the tissue during the recovery period may have had long-term effects.

An assessment of the toxicity of a compound to aquatic organisms must take into account environmental factors which affect its toxicity. Water conditions such as temperature, pH, salinity, dissolved oxygen, and hardness are known to affect the toxicity of pentachlorophenol to aquatic organisms. The presence of other toxicants may also affect the acute toxicity of pentachlorophenol in aqueous solutions.

Pentachlorophenol is almost certainly bioconcentrated in many aquatic organisms. Tissue levels 100 to 1000 times the level of ambient pentachlorophenol in water have been found in fish. The eel, a species with a high fat content, acquires higher levels of pentachlorophenol than fish species with a lower fat content. It is not known whether toxicologically significant amounts of pentachlorophenol may be accumulated by aquatic organisms over their lifetimes, particularly following intermittent exposures. Biomagnification (i.e., the presence of a substance in increasing concentrations at higher trophic levels in the food chain) has not been demonstrated conclusively for pentachlorophenol; however, evidence indicates that pentachlorophenol may be biomagnified in aquatic ecosystems. Model aquatic ecosystems have indicated that the mosquito fish (the highest trophic level in an experimental six-element food chain) accumulated pentachlorophenol at levels 300-fold higher than the level in the ambient environment. Other organisms in the food chain accumulated lower amounts which corresponded roughly to their trophic level. The hazard posed by biomagnification of pentachlorophenol in a natural aquatic system has not been elucidated. Pentachlorophenol may also appear in aquatic organisms as a result of degradation of hexachlorobenzene. One experiment has demonstrated the metabolism of hexachlorobenzene to pentachlorophenol in aquatic organisms, which indicates the possibility that pentachlorophenol in water may result from detoxification of hexachlorobenzene (or perhaps other compounds).

Fish can detect pentachlorophenol and demonstrate an avoidance reaction to it when placed in a pentachlorophenol gradient. This phenomenon is species dependent; levels of 100 μg/liter can be detected by the juvenile Atlantic salmon. The environmental significance of this ability is unknown.

Pentachlorophenol can cause acute systemic toxicity in humans following absorption through the respiratory tract, the gastrointestinal tract, or the skin. Minimum lethal concentrations for pentachlorophenol in air have not been defined; however, a number of documented cases of pentachlorophenol toxicity resulting in human death are believed to have had a respiratory component. Severe general upper respiratory distress usually accompanies human exposure to atmospheric pentachlorophenol; some tolerance is found in individuals who routinely handle the material. Despite the characteristic warning of exposure to atmospheric pentachlorophenol (i.e., respiratory distress), one clear-cut case of respiratory pentachlorophenol poisoning has been reported. The victim occupied a house whose interior redwood siding had been improperly treated with the preservative. Because pentachlorophenol is not likely to be present as a dust or an aerosol, pentachlorophenol vapor was believed to be responsible. The small, but substantial, vapor pressure of pentachlorophenol may allow toxic levels of the compound to build up in hot, enclosed areas. Studies with experimental animals as well as several reports of human systemic toxicity following ingestion of pentachlorophenol have shown that the compound is rapidly absorbed through the gastrointestinal tract. Ingestion of pentachlorophenol is probably rare among humans. Only in such exceptional circumstances as suicides or extraordinary insensitivity is a person likely to ingest a compound of such noxious character. However, possible ingestion of small amounts of pentachlorophenol in the diet cannot be ignored.

Most reported cases of pentachlorophenol intoxication in humans involve direct absorption of pentachlorophenol or its sodium salt through the skin. Absorption through the skin following contact may be extremely rapid, with severe symptoms and death occurring after very short (a few minutes) exposures. The nature of the solvent in which pentachlorophenol is dissolved apparently affects uptake and subsequent toxicity of the compound, but no investigations of why the organic solvent affects cutaneous absorption were found. Pentachlorophenol appears to be most toxic when applied in petroleum solvents and is less toxic in vegetable oils. Following cutaneous application, pentachlorophenol in organic solvents seems to be considerably more toxic to experimental animals than sodium pentachlorophenate in aqueous solution. Repeated application of soap and water is more effective in removing pentachlorophenol or sodium pentachlorophenate from the skin than alcohol application.

Whether pentachlorophenol is absorbed through the skin or respiratory tract or is ingested, it is distributed in and transported by the blood. Pentachlorophenol apparently is not bound to the cellular constituents in blood, but evidence exists that pentachlorophenol may bind to plasma proteins. Studies performed with experimental animals indicate that pentachlorophenol is present in almost all tissues within 24 hr following a lethal oral dose. However, the highest levels of pentachlorophenol in experimental animals or in lethally poisoned humans have been found in the liver, stomach, intestines, kidney, gallbladder, blood, and urine. The compound may be present in the kidney because of the function of this organ in urinary excretion. Its presence in the liver may arise from pentachlorophenol detoxication; high pentachlorophenol levels in the gallbladder, stomach, and intestines indicate that it may be present in gastric and biliary secretions that cause fecal excretion.

The extent to which pentachlorophenol transfers across the placenta in mammals has not been adequately defined. Experiments have yielded results that are difficult to interpret quantitatively, and more research in this area is warranted.

Metabolic alteration of pentachlorophenol in mammals and humans has been studied. In mice, free pentachlorophenol and its conjugates and tetrachlorohydroquinone and its conjugates are excreted in the urine. Chloranil, a compound closely related to tetrachlorohydroquinone, also has been identified in the urine of experimental animals dosed with pentachlorophenol. In only one case has a pentachlorophenol conjugate, the glucuronate, been identified in mammals. Biotransformation of pentachlorophenol may take place in the liver, but no direct information is available on the tissue sites for metabolism of pentachlorophenol.

In humans and experimental animals, pentachlorophenol appears to be excreted primarily through the urine. Although initial elimination may be rapid, a return to background levels may take longer than one month. There are species differences in kinetics and metabolites. Approximately 50% of the administered pentachlorophenol is excreted in the urine in 24 hr, and 70% to 85% is excreted in four days. Pentachlorophenol levels in the urine correspond to the quantity of the compound absorbed. Thus, urinary pentachlorophenol levels have been used as a diagnostic tool in determining pentachlorophenol exposure in humans. In view of the data obtained from experimental animals, a true assessment of pentachlorophenol exposure should include examination of the urine for the commonly seen metabolic products of pentachlorophenol metabolism, including pentachlorophenol conjugates, tetrachlorohydroquinone and its conjugates, and chloranil. The presence of these compounds would indicate biodegradation of pentachlorophenol in the organism. Fecal excretion also has a role in elimination. Values ranging from 4% of the injected dose in the rabbit to 13% in rats over a ten-day period have been reported, but this type of information is rare. There is evidence of enterohepatic circulation.

The primary mechanism of pentachlorophenol toxicity in humans is likely its ability to uncouple oxidative phosphorylation and to short-circuit metabolism. An increase in cellular respiration and the generation of considerable heat accompany the uncoupling of oxidative phosphorylation. Effects on specific organs as a result of the disruption of normal levels of ATP are speculative, but massive damage occurs if these vital processes within the cell are disrupted to any large extent.

Both pentachlorophenol and sodium pentachlorophenate can cause local inflammation on skin contact. Damage ranges from minor irritation and redness to edema, inflammation, and necrosis of skin, depending on the concentration of the solution and the type of carrier solvent. A serious skin disease known as chloracne can result from long-term cutaneous contact with pentachlorophenol or sodium pentachlorophenate. It is not clear whether pentachlorophenol or impurities such as chlorodibenzo-p-dioxins commonly found in technical-grade pentachlorophenol formulations are responsible for chloracne in humans.

Acute systemic toxicity due to absorption of pentachlorophenol or sodium pentachlorophenate results in major degenerative changes in vital organs, including the gastrointestinal tract, lungs, spleen, liver, kidneys, and heart. Symptoms exhibited by humans acutely poisoned by pentachlorophenol are weight loss, general weakness, fatigue, dizziness, mental weakness, headache, anorexia, nausea and vomiting, dyspnea, hyperpyrexia, respiratory distress, tachycardia, hepatomegaly, profuse perspiration, and elevated basal metabolic rate. The most common symptoms of pentachlorophenol intoxication are general weakness, weight loss, and profuse perspiration. About 50 cases of poisoning from pentachlorophenol ingestion or absorption have been reported and 30 resulted in death. No specific antidote for the poison is known, and the deaths occurred despite conventional supportive therapy. Infants poisoned by cutaneous contact with pentachlorophenol were successfully treated with exchange transfusions. The use of intravenous fluids to promote renal excretion may be beneficial.

Susceptibility to pentachlorophenol poisoning may depend on factors such as the capacity of the renal system to handle the prevalent load of pentachlorophenol and the patient's general health. The exact dosage of pentachlorophenol required to produce illness in humans is not known. Symptoms occur at pentachlorophenol concentrations of 40 to 80 mg/liter in the blood; blood levels noted in fatal cases ranged from 46 to 156 mg/liter, and urine levels ranged from 28 to 520 mg/liter. The oral lethal dose for humans has been listed as 29 mg/kg body weight. However, as noted before, the actual amounts required to cause systemic toxicity probably depend on the renal competency and general health of the individual. Another factor which may influence pentachlorophenol toxicity is high ambient temperature. Most fatal cases of pentachlorophenol poisoning resulted from exposure when ambient temperatures were high. Because the primary effect of pentachlorophenol intoxication is hyperpyrexia, increased toxicity of pentachlorophenol with high ambient temperatures may be due to increased difficulty in dissipating excess body heat generated by the uncoupling of oxidative phosphorylation.

Chronic pentachlorophenol poisoning of experimental animals with extremely low doses has not been clearly demonstrated. No chronic systemic disease was noted in rabbits when pentachlorophenol was applied cutaneously at levels low enough to avoid gross skin damage. Daily subcutaneous or intraperitoneal doses of pentachlorophenol to rabbits may cause severe symptoms and death if the doses are high enough. The amount of pentachlorophenol necessary to cause death when it is administered in small, divided doses is very similar to the minimum lethal dose for a single administration of the compound. Renal competency may determine the chronic levels of pentachlorophenol which may be tolerated. Thus, cases of chronic poisoning may result not from a storage-type accumulation of the compound in the system, but rather from uptake in excess of excretion rate (which is determined by the ability of the renal system to handle the prevalent load). A steady state may be reached when levels of pentachlorophenol are high enough to cause systemic intoxication. Chronic effects from storage-type accumulation of pentachlorophenol in experimental animals have not been demonstrated. Rats fed diets containing pentachlorophenol in concentrations too low to cause severe systemic effects had a dose-related decrease in calcium deposits in the kidney. This effect is not well understood, and its importance to the well-being of the organism is unknown. Chronic effects from storage-type accumulation of pentachlorophenol in experimental animals have not been demonstrated.

Low-dose pentachlorophenol exposure may not result in chronic effects in humans. Although low but detectable levels of pentachlorophenol have been found in the blood and urine of occupationally and nonoccupationally exposed persons, no chronic effects from these prevalent levels have been noted. Chronic exposure of persons in the wood treatment industry to pentachlorophenol reportedly has reversible effects on kidney function. Investigators have concluded that pentachlorophenol exposure results in decreased creatinine clearance and phosphorus reabsorption in the kidney but that the effect is reversible. Significant differences in blood and urinary phosphorus levels and in creatinine clearance have been found in wood treaters before, during, and after vacation. Such minor effects on kidney function are probably in most cases insignificant; however, the question warrants further study. Workers chronically exposed to pentachlorophenol may also show a significantly higher prevalence of gamma-mobility C-reactive protein in the sera. Although the clinical significance of these elevated levels is not known, C-reactive protein levels are often elevated in acute stages of inflammation or tissue damage. These data have not been verified.

Pentachlorophenol may accumulate in adipose tissue of humans. Levels of 12 to 45 μg/kg tissue have been found in individuals not occupationally exposed to pentachlorophenol. The levels of pentachlorophenol in fat are likely toxicologically insignificant. Increased levels may be found in individuals occupationally exposed to the compound. The potential for biomagnification in human adipose tissue is an open question warranting further investigation.

The almost ubiquitous presence of pentachlorophenol in humans may result from the metabolism of other organochlorine compounds. For example, pentachlorophenol has been isolated from the excreta of rats and monkeys following administration of lindane. This finding causes question of the tacit assumption that pentachlorophenol in human urine, serum, and tissue results from direct exposure. In almost all cases of pentachlorophenol poisoning in humans (fatal and nonfatal), misuse of the compound is paramount. Industrial safety records for pentachlorophenol handling are excellent, but deaths of casual or inexperienced users and other nonindustrial fatalities resulting from inappropriate use of the compound (e.g., death of infants in a St. Louis nursery where pentachlorophenol was inappropriately used in laundry facilities) have been reported. Adequate protection from cutaneous or respiratory contact with pentachlorophenol should prevent most cases of human systemic intoxication. When explicit safety precautions are not noted, common sense would indicate that exposure to the compound should be minimized.

Market basket studies conducted by the U.S. Food and Drug Administration have shown that some portion of the nation's population is exposed to low levels of pentachlorophenol through food consumption. The sources of the residues in food are not known, and the hazards associated with consumption of these small quantities of pentachlorophenol remain speculative.

Pentachlorophenol does not promote tumor formation in mice following oral administration or following cutaneous application of a single initiating dose of dimethylbenzanthracene. Other chlorophenol compounds and phenol possess tumor-promoting activity following cutaneous application of dimethylbenzanthracene. Also, pentachlorophenol did not exhibit mutagenic properties when it was tested in a microbiological system, in a mammalian test system, and in the fruit fly (*Drosophila melanogaster*). Pentachlorophenol does not appear to be teratogenic but is fetotoxic. Delayed development has been observed when high pentachlorophenol dosages are administered to the maternal rat. However, fetotoxic effects likely result from a direct effect on the maternal rat. The most prominent effect of pentachlorophenol on the mammalian system is hyperpyrexia, and this alone may cause the fetotoxicity.

Monitoring data for levels of pentachlorophenol in the atmosphere are unavailable, but circumstantial evidence indicates that pentachlorophenol may be present in the atmosphere. Levels of pentachlorophenol in rainwater and snow samples taken from the Mauna Kea Summit in Hawaii indicated that pentachlorophenol is likely present in the atmosphere either as a vapor or as an occlusion on dust particles and is removed from the atmosphere by washout. Significant amounts of pentachlorophenol are discharged in gaseous and particulate forms from manufacturers of pentachlorophenol. Detailed information on pentachlorophenol levels in the atmosphere surrounding such plants is not available.

Monitoring data on the presence of pentachlorophenol in the aquatic environment are scant. There have been numerous reports of fish kills following industrial accidents, including spillage and seepage of concentrated pentachlorophenol. Deleterious effects on aquatic organisms following the widespread distribution of pentachlorophenol as a molluscicide or algicide have also been reported. However, these lethal occurrences are usually associated with the presence of high levels of pentachlorophenol in water. Documentation of low-level pentachlorophenol contamination of water is not available. Measurable amounts of pentachlorophenol are present in treated and untreated wastewaters from wood treatment plants. Pentachlorophenol has also been detected in storm water draining industrial sites where treated lumber has been stored, and its presence has been confirmed in municipal sewage. Furthermore, measurable pentachlorophenol levels have been detected in the Willamette River in Oregon and in the drinking water supplies of Tallahassee, Florida, and Corvallis, Oregon. Contamination sources could include leaching from preservative-treated items; use of pentachlorophenol as a slimicide, as an algicide, or, more infrequently, as a herbicide; inadequate treatment of sewage containing pentachlorophenol; synthesis during drinking water or wastewater chlorination procedures; and synthesis from other related organic compounds such as hexachlorobenzene.

The persistence of pentachlorophenol in aquatic environments depends on a number of environmental variables; the interrelationships have not been fully characterized. Pentachlorophenol may be dissipated from water by volatilization, photodecomposition, sorption, or biodegradation. The primary mode of removal is likely biodegradation. Decomposition of pentachlorophenol in aqueous solution has been demonstrated under laboratory conditions in the presence of suitably activated microbial populations. In the laboratory, microorganisms contained in activated sludge derived from sewage treatment plants exposed to pentachlorophenol are capable of degrading the compound. However, the distribution of these microorganisms in the environment is unknown. Under specified conditions, pentachlorophenol levels in water declined to negligible amounts in 48 hr; other experiments indicated that substantial amounts of pentachlorophenol may remain in a laboratory system for more than 120 days. Thus, depending on environmental conditions, pentachlorophenol may degrade rapidly and pose little or no environmental hazard, or it may have marked longevity and thereby pose a potential hazard of indeterminate proportions.

Ambient levels of pentachlorophenol in soil are not available. Possible sources of contamination include direct application as a herbicide or desiccant, washout from the atmosphere, and leaching from preservative-treated wood. Mobility and persistence of pentachlorophenol depend on the physical and chemical characteristics of the soil as well as on the prevailing microbial population. Pentachlorophenol is strongly sorbed in most soils, and movement through the soil is likely to be negligible. A large number of environmental parameters such as temperature; pH; moisture; organic matter, clay mineral, and free iron contents; sorption characteristics; cation-exchange capacity; and microbiological composition affect the persistence of pentachlorophenol in soils. Microbiological activity plays a primary role in the degradation of pentachlorophenol in soil, and persistence ranges from 21 to 200 days, depending on specific conditions at the site. Apparently, acclimated microbial populations (those previously exposed to pentachlorophenol) facilitate pentachlorophenol decomposition in soil. Under conditions unfavorable for bacterial growth, pentachlorophenol degradation is extremely slow.

Pentachlorophenol is considered to be a refractory compound of the chlorophenol family. Although microbial populations capable of rapid pentachlorophenol degradation have been isolated, gradual acclimation of the bacteria to increasing pentachlorophenol levels is required to produce strains which optimally degrade pentachlorophenol. In many cases where rapid pentachlorophenol degradation was noted without initial acclimation of the bacteria to pentachlorophenol, the initial microbial inoculum was frequently derived from areas where pentachlorophenol had been used for a considerable time. In the presence of alternative substrates, pentachlorophenol-degrading bacteria may not be optimally efficient. Other bacterial populations are capable of preferentially degrading pentachlorophenol in the presence of an alternative carbon source. It is not known whether these bacteria possess qualities which allow them to compete effectively in a mixed population. Data indicate that pentachlorophenol-degrading bacteria are present in soil surrounding pentachlorophenol-treated wood. In general, pentachlorophenol is moderately stable in preservative-treated wood and dissipates slowly as a result of biodegradation and leaching. The environmental hazard of properly treated wood is probably minimal. Slow contamination, when it occurs, is likely confined to very localized areas surrounding the treated wood, and the pentachlorophenol which reaches the soil is degraded sufficiently rapidly to prevent a hazard to plant and animal life. However, in light of the extremely high toxicity of pentachlorophenol to aquatic organisms and possible biomagnification, the use of pentachlorophenol-treated wood in or around aquatic environments should be carefully regulated.

Evidence exists that pentachlorophenol-containing effluent can be degraded in an aeration lagoon. Based on one laboratory study, the aeration lagoon apparently provides an efficient method for the secondary treatment of pentachlorophenol-containing wastewater. In the laboratory, activated sludge populations are capable of degrading pentachlorophenol, but retention times are quite long because pentachlorophenol is a relatively stable molecule. The efficiency of these processes in any given sewage treatment plant must be assessed on an individual basis. When adequate precautions are taken and retention times are long enough, adequate biological elimination of pentachlorophenol from wastewater can likely be achieved by biodegradation. Of more serious concern is the disruption of normal sewage treatment processes when the compound is not a regular component of the influent wastewater.

D.1.2 CONCLUSIONS

1. The most widely used, effective technique for analysis of pentachlorophenol in many sample types is gas-liquid chromatography.

2. Pentachlorophenol is extremely toxic to almost all forms of bacteria, algae, and fungi and is widely used as an antimicrobial agent and as a preservative.

3. Pentachlorophenol is rapidly absorbed by the roots and foliage of vascular plants but does not translocate readily to other plant parts.

4. Vascular plants (terrestrial and aquatic) are extremely sensitive to the toxic effect of pentachlorophenol, and contact with crops or ornamental plants should be scrupulously avoided.

5. Exposure of wild or domestic animals to concentrated pentachlorophenol solutions or their constant cutaneous exposure to pentachlorophenol-treated wood may result in illness or death.

6. Current data are insufficient to evaluate chronic long-term effects of pentachlorophenol-treated wood on livestock. Preservative-treated wood probably poses no serious hazard to domestic animals if the wood has been properly treated and if animals do not have constant cutaneous contact with the treated wood.

7. The toxic effect of pentachlorophenol probably lies in its ability to uncouple oxidative phosphorylation in living organisms, thereby short-circuiting metabolism.

8. Pentachlorophenol biotransformation in animals and humans probably involves the formation of pentachlorophenol conjugates (including glucuronides), tetrachlorohydroquinone, tetrachlorohydroquinone conjugates, and chloranil.

9. Extensive loss of wildlife has been reported following the application of large amounts of pentachlorophenol as a molluscicide; such usage is not recommended.

10. Pentachlorophenol is extremely toxic to aquatic organisms; sensitive species of fish perish at concentrations as low as 30 μg/liter. Measurable sublethal effects from chronic exposure have been documented at concentrations less than 2 μg/liter.

11. Following uptake of pentachlorophenol by fish, initial elimination is very rapid. However, approximately 20% of the pentachlorophenol absorbed may remain in fish tissue for an extended time; storage in fat may account for this phenomenon.

12. Pentachlorophenol is almost certainly bioconcentrated in many aquatic organisms; tissue levels 100 to 1000 times the ambient level of pentachlorophenol in water have been found in fish.

13. Pentachlorophenol may be subject to biomagnification in aquatic ecosystems (i.e., found in increasing concentrations at higher trophic levels in the food chain).

14. In view of its high toxicity to aquatic animals and the likelihood of bioconcentration, low limits for pentachlorophenol residues in waterways should be considered.

15. Appearance of pentachlorophenol in aquatic organisms and experimental mammals following absorption of hexachlorobenzene has been documented.

16. Nearly all reported cases of severe systemic pentachlorophenol poisoning in humans involved either poor hygiene or improper usage of the compound.

17. Following uptake of pentachlorophenol by humans or experimental animals, high levels are found in the liver, stomach, intestines, kidney, gallbladder, blood, and urine, with the highest levels in the gallbladder. Possibly, the gallbladder could serve as an indicator organ in establishing pentachlorophenol exposure.

18. Placental transfer of pentachlorophenol is controversial.

19. Data from experimental animals indicate that a true assessment of human exposure to pentachlorophenol should include examination of the urine for the commonly seen metabolic products of pentachlorophenol metabolism (pentachlorophenol conjugates, tetrachlorohydroquinone, tetrachlorohydroquinone conjugates, and chloranil).

20. Although initial elimination may be rapid following human exposure to pentachlorophenol, a return to background levels may take more than a month. Inadequate experimental methods preclude definitive conclusions, and carefully controlled studies are needed.

21. Low, but detectable, levels of pentachlorophenol have been found in blood, urine, and fat of nonoccupationally exposed persons, but chronic effects from these prevalent levels have not been demonstrated.

22. Minor effects on kidney function and a significantly higher prevalence of gamma-mobility C-reactive protein in the sera have been associated with chronic pentachlorophenol exposure in workers in the wood treatment industry, but the significance of these effects to the health of the individuals is speculative.

23. Available data indicate that pentachlorophenol does not possess tumorigenic, mutagenic, or teratogenic properties.

24. Circumstantial evidence indicates that pentachlorophenol is present in the atmosphere.

25. Low levels of pentachlorophenol have been detected in river water and in several municipal water supplies; the health hazard posed by these levels is unknown.

26. Pentachlorophenol is rapidly dissipated in the aquatic environment when suitably acclimated microbial populations are present; under less than ideal conditions, however, pentachlorophenol persistence may be considerably extended.

27. Pentachlorophenol is strongly sorbed in most soils, and movement through the soil profile is unlikely; persistence ranges from 21 to 200 days, depending on specific conditions at the site.

28. Acclimated microbial populations (those previously exposed to pentachlorophenol) facilitate decomposition in soil. Under conditions where bacterial growth is not favored, pentachlorophenol degradation is extremely slow.

29. The environmental hazard of wood properly treated with pentachlorophenol is probably minimal in the terrestrial environment.

30. The use of pentachlorophenol or pentachlorophenol-treated wood in or around aquatic environments should be carefully examined because of its high toxicity to aquatic organisms and possible biomagnification in aquatic ecosystems.

31. Pentachlorophenol can be readily degraded in aeration lagoons or activated sludge when suitably acclimated microorganisms are present and retention times are of sufficient length.

32. The presence of low quantities of pentachlorophenol in foods consumed by humans has been reported, but health hazards associated with consumption of foods containing low levels of pentachlorophenol have not been documented.

D.2 CHEMICAL AND PHYSICAL PROPERTIES AND ANALYSIS

D.2.1 PHYSICAL PROPERTIES

In the pure state, pentachlorophenol is a white, needlelike, crystalline solid. Because it is practically insoluble in water, its sodium salt, which is readily soluble in water, is substituted for many practical uses. Some physical properties of pentachlorophenol are listed in Table A.2.1.

D.2.2 MANUFACTURE

Pentachlorophenol is synthesized commercially by the direct chlorination of phenol. Chlorination proceeds stepwise, and catalysts such as $FeCl_3$, $AlCl_3$, and $SbCl_3$ are used in concentrations of 0.05% to 1.0%. Usually, the reaction is carried out in the absence of solvents (with direct addition of gaseous chlorine to molten phenol), but carbon tetrachloride, acetic acid, and water have been used as diluents. In industry, the starting material for pentachlorophenol production may be a mixture of various chlorophenol isomers if these compounds are readily available (Doedens, 1963). Pentachlorophenol may also be produced through hydrolysis of hexachlorobenzene, but this method is less popular than the direct chlorination technique.

D.2.3 USES

Pentachlorophenol and sodium pentachlorophenate are used extensively as broad-spectrum biocides. In 1972, annual production in the United States exceeded 50,000,000 lb (23,000,000 kg) (Anonymous, 1972). Growth was projected at about 4% per annum after 1972. Estimated production of pentachlorophenol and sodium pentachlorophenate in 1976 was 56,000,000 lb (25,400,000 kg).

Pentachlorophenol (or sodium pentachlorophenate) has been used as a wood preservative to control mold and termite infestations (Fuller et al., 1977), as a weedicide or preharvest desiccant, as an algicide, as a molluscicide for the eradication of the snail which serves as an intermediate host for the schistosomes in humans, and in food processing plants and pulp mills for the control of slime and mold. Pentachlorophenol and sodium pentachlorophenate also have been used as fungicides and/or bactericides in the processing of cellulosic products, starches, adhesives, paints, leathers, oils, rubber, textiles, and wooden crates used for packaging raw agricultural products. Obviously, a product with such widespread and varied uses poses a serious potential for environmental contamination (Bevenue and Beckman, 1967).

D.2.4 CHEMICAL REACTIVITY

Pentachlorophenol is quite stable; it does not decompose when heated at temperatures up to its boiling point for extended periods. Pure pentachlorophenol is considered to be rather inert chemically

(Bevenue and Beckman, 1967). The chlorinated ring structure tends to impart stability, but the polar hydroxyl group facilitates biological degradation (Renberg, 1974). Pentachlorophenol is not subject to the easy oxidative coupling or electrophilic substitution reactions common to most phenols. Any monovalent alkali metal salt of pentachlorophenol is very soluble in water, but the protonated (phenolic) form is virtually insoluble. Hence, transport of pentachlorophenol in water depends largely on the pH of the environment.

Pentachlorophenol is volatile enough to be steam distilled — a property that can be exploited by the analyst. If a closed system is not used when environmental samples are heated, recoveries are poor (Bevenue and Beckman, 1967). By analogy to other chlorinated organic compounds with low vapor pressures, volatility may cause significant losses of pentachlorophenol from soils (Briggs, 1975).

D.2.5 TRANSPORT AND TRANSFORMATION IN THE ENVIRONMENT

The transport and transformation mechanisms of pentachlorophenol in air, soil, and aquatic environments are discussed fully in Section D.7. These mechanisms are summarized in this section to give a perspective on the possible relationships of the physical and chemical properties of the compound to its environmental interactions.

D.2.5.1 Air

Pentachlorophenol can enter the atmosphere by several routes. Even though its vapor pressure is low, a finite amount will vaporize. In the past, use of pentachlorophenol as a spray-on herbicide released the material directly into the air. Also, depending on the solvent system and conditions, pentachlorophenol may recrystallize on the surface of treated wood (referred to as blooming), and these small crystals can then be brushed off into the ambient air. The significance of any of these routes is determined by the specific circumstances. Volatilization is likely the major mechanism for dispersal of the compound into the atmosphere; however, monitoring data are lacking.

D.2.5.2 Aquatic Environment

In the aquatic environment, pentachlorophenol may be in dissolved form, associated with suspended matter or bottom sediments, or absorbed by organisms. Metal salts of the compound have much greater water solubility and therefore would exist primarily in the dissolved form. The tendency of pentachlorophenol to ionize depends on the pH of the system. It is nonionized in aqueous solutions with pH lower than 5 and becomes increasingly dissociated as the pH rises. The degree of dissociation could determine the extent of sorption of colloids present in aquatic systems; however, specific information is not available. Hydrological factors such as current patterns and mixing as well as sorption, degradation, and migration of organisms affect the movement of the chemical.

There is limited evidence of microbiological degradation of pentachlorophenol in aquatic environments. Photodecomposition and volatilization from water also occur. Detailed discussions of these phenomena are found in Section D.7.

D.2.5.3 Soils

One of the most important environmental aspects of a synthetic organic chemical is its interaction with the soil. Soil is the ultimate absorber and purifier of many of the most toxic chemicals. In the soil, pentachlorophenol is affected by many complex factors; many details of its behavior are unknown or have not been verified (Bevenue and Beckman, 1967). Greenhouse studies of soils treated with sodium pentachlorophenate showed that the toxicity of the compound to sweet corn and cucumber decreased with time; toxicity was greatest at higher (approximately 45°C) temperatures (Bevenue and Beckman, 1967). The sodium salt was stable in air-dried soil; it persisted for two months in soil with a moderate moisture content and for only one month is water-saturated soil. It seemed to be more stable in heavy-clay soils than in sandy soils (Bevenue and Beckman, 1967).

Hilton and Yuen (1963) studied the adsorption behavior of pentachlorophenol in several Hawaiian soils growing sugar cane. They compared its adsorption with that of a variety of substituted urea herbicides — linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea], diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and monuron [3-(*p*-chlorophenyl)-1,1-dimethylurea] — and with substituted triazine herbicides — atrazine [2-chloro-4-ethylamino-6-(isopropylamino)-*s*-triazine] and simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine]. Adsorption of pentachlorophenol was the highest of all the compounds studied. A study with ten soils showed no relationship between adsorption and soil pH. Good weed control is achieved only in soils with low adsorptivity. Excessive rates of application (up to 500 kg/ha) provided only slightly better weed control than 50 kg/ha. The steep slopes of the adsorption isotherms for pentachlorophenol indicate that large increases in pentachlorophenol application are required to produce a significant increase in the soil solution concentration of pentachlorophenol.

Dobrovolny and Haskins (1953) studied the effects of different soils on adsorption of dilute concentrations of sodium pentachlorophenate. Greater depth of the mud layer in proportion to depth of the water layer produced a more rapid decrease in the sodium pentachlorophenate content of the water layer. As expected, sand did not adsorb sodium pentachlorophenate. The nature of the chemistry between the sodium pentachlorophenate and the clay or silt in the sediments was not investigated.

Choi and Aomine (1972, 1974*a*, 1974*b*) have studied the interactions of pentachlorophenol and soil extensively. Adsorption of pentachlorophenol at various concentrations was studied in several different soils (Choi and Aomine, 1972). After adsorption equilibrium was attained, the concentration of pentachlorophenol in the supernatant solution was measured by the 4-aminoantipyrine method. In addition, a bioassay was conducted with wheat seedlings to measure the inhibitory rate of L_{50} defined as:

$$\frac{\text{length of untreated seedlings in control plot - length of pentachlorophenol-treated seedlings}}{\text{length of untreated seedlings}} \times 100.$$

The L_{50} represents the pentachlorophenol concentration at which the length of treated seedlings is 50% of that of the untreated seedlings; this value is determined by graphing seedling length against concentration of pentachlorophenol. Adsorption and/or precipitation of pentachlorophenol occurred to some extent in all soils tested. The bioassay revealed that inhibition of the wheat seedlings was greater for the soil suspension than for the supernatant aqueous solution above it; however, the inhibitory rate of the soil suspension was less than that of the standard pentachlorophenol solution. These findings indicate that adsorbed and/or precipitated pentachlorophenol retained some toxicity to the plant.

Choi and Aomine (1974*a*) also studied adsorption behavior by using 13 soil samples which had various clay mineral species, organic matter content, and pH and seven different concentrations of pentachlorophenol ranging from 12.5 to 500 mg/liter. They concluded that adsorption behavior depends primarily on the pH of the system; the more acid the soil, the more complete is the "apparent adsorption" of pentachlorophenol. Different mechanisms of adsorption dominate at different pH values. In acid clays, "apparent adsorption" involves adsorption on colloids and precipitation in the micelle and in the external liquid phase. Organic matter content of the soil is important to "apparent adsorption" of pentachlorophenol at all pH values. At different pH values, comparisons of untreated soil and soil oxidized with hydrogen peroxide (H_2O_2) to remove organic matter showed that humus-containing soil always adsorbed more pentachlorophenol than H_2O_2-treated soil. The investigators assumed that H_2O_2 oxidation and extraction with an organic solvent removed only organic matter and that this did not change the structure or adsorption characteristics of the inorganic fraction remaining; however, they realized that these assumptions probably were incorrect. Later investigations led to the conclusion that adsorption of pentachlorophenol by humus is important when the concentration of pentachlorophenol is low (i.e., when pentachlorophenol is adsorbed largely in the undissociated state). At higher concentrations of pentachlorophenol, the inorganic fractions become more important.

D.2.5.3.1 Effect of Temperature on Pentachlorophenol Adsorption by Soil — Choi and Aomine (1974*b*) studied the effects of temperature on adsorption of pentachlorophenol by soil. Four allophanic soils with pH adjusted to 5.6 by addition of NaOH or HCl were used to measure the adsorption of pentachlorophenol at 4°C and 35°C. Three soils showed a significant increase in pentachlorophenol adsorption at higher temperatures, but the fourth soil showed a decrease. The authors explained the results on the basis of differences between the soils. They assumed that andosols (molar SiO_2/AlO_3 ratio of approximately 1) chiefly adsorbed pentachlorophenol as anions and that the major factor influencing pentachlorophenol adsorption by the one soil showing a decrease with increasing temperature (molar SiO_2/AlO_3 ratio of about 2) was a van der Waals' force.

D.2.5.3.2 Effect of Anions on Pentachlorophenol Adsorption by Soil — Choi and Aomine (1974*a*) studied pentachlorophenol adsorption in three different soils in the presence of chloride and sulfate ions. They adjusted 5 g of air-dried soil to pH 5.6 and added 60 mg/liter pentachlorophenol solution to the soil samples. Because the pH of the soil suspension was lowered by adding sodium salts (the counterion for the chloride and sulfate), the amount of NaOH needed to maintain the pH at 5.6 increased with increasing concentrations of added salts. Total volume of liquid was adjusted to 50 ml. At lower salt concentrations, more pentachlorophenol was adsorbed by the soil samples. The allophanic soils showed greater adsorption of pentachlorophenol in the presence of NaCl than in the presence of Na_2SO_4, but the soil with a higher SiO_2/AlO_3 ratio (approximately 2) did not exhibit this trend. These results indicate competition between the inorganic anions and the pentachlorophenol anions for adsorption sites on the soil colloid. As would be expected, sulfate ions are able to compete with pentachlorophenol for allophanic adsorption sites more effectively than are chloride ions.

D.2.5.3.3 Influence of Ions on the Allophane Surface on Pentachlorophenol Adsorption — Allophanic clay separated from soil was used to study the effect of surface charge on pentachlorophenol adsorption. Choi and Aomine (1974*b*) treated clay samples either with a 1 *N* sodium acetate buffer solution at pH 3.5 or with 2% Na_2CO_3. The Na_2CO_3 treatment was more effective in reducing surface acidity than was the acetate buffer treatment, and sodium acetate-treated clay displayed more positive charge than Na_2CO_3-treated clay (Figure D.2.1). The adsorption of pentachlorophenol on soil apparently involved anion-exchange reactions as well as physical adsorption due to van der Waals' forces.

Pentachlorophenol mixed with layered silicate clay minerals such as illite, montmorillonite, and kaolinite sublimed at about 200°C. Pentachlorophenol mixed with or adsorbed on allophane did not sublime at 200°C but pyrolized between 250°C and 500°C, showing a strong exothermic reaction (Choi and Aomine, 1974*b*).

D.2.6 ANALYTICAL METHODS

In determining pentachlorophenol in soil, water, or biological materials, the validity of results is assured only when the sample is representative and when the pentachlorophenol in the sample is identified and measured accurately. In complex samples such as soil, water, and sediments, the investigator must consider the unknown or complex relationships affecting representative sampling. Compromise must often be reached between the best method of sampling and the funding available.

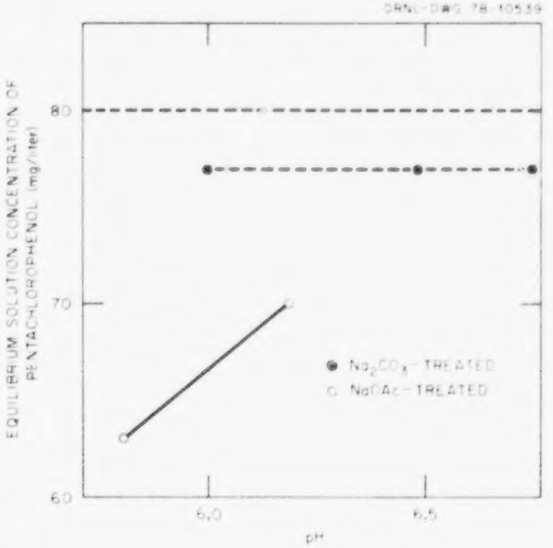

Figure D.2.1. Effect of pH on the adsorption of pentachlorophenol by isolated allophanes. Source: Adapted from Choi and Aomine, 1974*b*, Figure 5, p. 377. Reprinted by permission of the publisher.

Given a representative sample, the performance of an analytical method depends on the quantitative extraction of pentachlorophenol and its accurate measurement and identification. The analyst must be careful that the method of determining extraction efficiency can be extrapolated to environmental samples and can provide uniformly quantitative extraction from a variety of samples. For water samples, pentachlorophenol distribution between extractant and water is important. For soil and sediment samples, the removal of pesticides from sorption sites is necessary (Chesters, Pionke, and Daniel, 1974). In biological tissues, separation from lipids must be achieved (Renberg, 1974). Also, because pentachlorophenol residues are found in a number of samples, including water, the analyst must be careful to exclude the possibility of sample or glassware contamination, especially when working with low-level samples.

The actual method of analysis must be sensitive enough to detect quantities of pentachlorophenol present and not be subject to interference from other compounds. Some methods (e.g., ultraviolet) require "cleaner" extracts and more elaborate cleanup prior to final determination. Gas-liquid chromatography has inherently great potential for separation of compounds so that rigorous cleanups are not necessary. For a detailed review of many other specific factors to be considered in evaluating analytical methods, see Chesters, Pionke, and Daniel (1974).

Many methods of qualitative and quantitative analysis have been developed for pentachlorophenol (Tables D.2.1 and D.2.2). Samples are sometimes expected to contain pentachlorophenol and no chemical analogs;

TABLE D.2.1. METHODS OF DETERMINATION OF PENTACHLOROPHENOL IN SEVERAL SAMPLE MATERIALS

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
Urine	Extracted with petroleum ether; reextracted with NaOH and acidified	Turbidimetric; 5 mg/liter	Rapid scanning, routine method	Comstock, Comstock, and Ellison, 1967
Human adipose tissue[a]	Extracted NaOH solution with hexane; acidified; extracted with ether; ethylated with diazoethane; pentachlorophenyl ethyl ether separated from hexachlorophene on a silica gel column	Electron-capture gas chromatography; 5 μg/kg	Method requires about 200 mg tissue	Shafik, 1973
Urine, blood, and air	Air samples collected with midget impingers using alkaline water or isooctane	Electron-capture gas chromatography	Air sampling procedure inadequately described	Casarett et al., 1969
Urine	Added NaOH; extracted with hexane; acidified; extracted with hexane	Electron-capture gas chromatography; 2 μg/liter	Seven alkyl ethers of pentachlorophenol used; several different columns used in detection	Cranmer and Freal, 1970
Natural latex	Coagulated with acetic acid; extracted with acetone	Thin-layer chromatography with $CuSO_4$-pyridine spray; 10 mg/g rubber	Semiquantitative, rapid method	Davies and Thuraisingham, 1968
Natural water[b]		Colorimetric using methylene blue or safranine O; 5 mg/liter	Hard water or water containing iron or copper yields interfering precipitates	Haskins, 1951
Natural water	Acidified and extracted into chloroform	Ultraviolet ratio spectrometry; 2 μg/liter	Other phenols do not interfere at chosen wavelength	Fountaine et al., 1975
Wood		Electron probe microanalyses; 0.1%	Determined distribution of pentachlorophenol in wood	Resch and Arganbright, 1971
Wood	Oxidized with chlorine dioxide	Microscopy; 0.022%	Determined distribution of pentachlorophenol in wood	Resch and Arganbright, 1971
Toy paints	Extracted with acetone	Flame-ionization gas chromatography with dansyl chloride derivatization; gas chromatography, 1 mg/liter; thin-layer chromatography, 4 mg/liter	Minimum background obtained by acetone extraction	van Langeveld, 1975

(continued)

TABLE D.2.1 (continued)

Sample	Isolation method	Analytical method and sensitivity	Remarks	Source
Water and sewage effluents	Extracted with benzene, followed by K_2CO_3 solution; acetylated and extracted with hexane	Electron-capture gas chromatography; 0.01 μg/liter	Acetylation in K_2CO_3 reduced interferences	Chau and Coburn, 1974
Soil, water, and fish	For soil and fish, extracted with KOH; acidified; extracted with toluene	Electron-capture gas chromatography of methyl ester; soil, 0.5 μg/kg; water, 0.01 μg/kg; fish, 0.5 μg/kg	Trimethylsilyl ether prepared for mass spectroscopy for confirmation	Stark, 1969
Biological tissue and water	Acidified; extracted with hexane; reextracted with borax solution	Electron-capture gas chromatography of ethyl ether; tissue, microgram per gram range; water, 0.01 μg/liter	Organochlorine insecticides did not interfere	Rudling, 1970
Blood, urine, tissue, and clothing	Extracted with ethyl ether; extracted ether solution with 5% NaOH; acidified; extracted with benzene	Electron-capture gas chromatography; 0.01 mg/liter or 0.01 μg/g		Barthel et al., 1969
Human blood	Acidified and extracted with benzene	Electron-capture gas chromatography of methyl ether; 20 μg/liter		Bevenue et al., 1968
Human urine	Acidified and extracted with petroleum ether	Electron-capture gas chromatography; picogram to nanogram range	No background interference	Bevenue et al., 1966
Biological samples	Acidified; extracted with ethyl ether; chromatographed on Celite-H_2SO_4 column; extracted with sodium pyrophosphate	Paper chromatography followed by uv spectroscopy; 5 μg/g	Laborious procedure	Erne, 1958

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[a] Sample contained pentachlorophenol and hexachlorophene.

[b] Sample contained sodium pentachlorophenate and copper pentachlorophenate.

TABLE D.2.2. METHODS OF DETERMINATION OF PENTACHLOROPHENOL IN SEVERAL SAMPLE MATERIALS [illegible]

Sample	Isolation method	Analytical method and sensitivity	Remarks	[illegible]
Urine	Acidified and extracted with petroleum ether	Gas chromatography–mass spectrometry; picogram to nanogram range	[illegible]	[illegible]
Natural water	Acidified and extracted with petroleum ether	Two-directional thin-layer chromatography with 4-aminoantipyrine or silver nitrate spray; [illegible] using silver nitrate; [illegible] using 4-aminoantipyrine	[illegible]	[illegible]
		Chromatography on Amberlite XAD resin using high-pressure liquid chromatography with [illegible]	[illegible]	[illegible]
Biological tissues and natural water	Steam distillation into NaOH	Colorimetric (nitric acid oxidation); 0.01 mg	[illegible]	[illegible]
Wood	Acetic acid/methanol extraction and adsorption on Bio-Rad AG2-X8 resin	Colorimetric 4-aminoantipyrine; [illegible]	[illegible]	[illegible]
		Thin-layer chromatography with dansyl chloride [illegible]	[illegible]	[illegible]
Fish tissue, soil, and natural water	Purification by binding acidic substances to anion exchanger	Electron-capture gas chromatography (derivatization); [illegible] 1.5 [illegible]/liter for water; [illegible] for soil	[illegible]	[illegible]
Fats, oils, waxes, and commercial food-grade fatty acids	Acidified; extracted with petroleum ether; extracted with NaOH; acidified and extracted with chloroform	Electron-capture gas chromatography; [illegible]	[illegible]	[illegible]
Biological tissues	Steam distillation and extraction with pentane or toluene	Electron-capture gas chromatography with [illegible]; [illegible] liter extracted [illegible]; 0.1 ng/g for [illegible]	[illegible]	[illegible]
	Acidified and extracted with isopropanol/hexane	Gas-liquid chromatography [illegible]; [illegible] range	[illegible]	[illegible]

methods for this type of sample are usually less sensitive and specific but more convenient than those used for residue analysis. The following publications were judged to present the best methods of analysis of those reviewed: Bevenue et al. (1968); Cranmer and Freal (1970); Rudling (1970); Buhler, Rasmusson, and Nakaue (1973); Frei-Häusler, Frei, and Hutzinger (1973); Renberg (1974); Zitko, Hutzinger, and Choi (1974).

Colorimetric and oxidation methods of analysis are less sensitive and specific than chromatographic methods. Attempts to modify the nitric acid oxidation method and the 4-amino antipyrine (4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) colorimetric method to improve selectivity and lower detection limits have not been very successful when only small amounts of sample are available (Bevenue and Beckman, 1967). Infrared or ultraviolet spectrophotometry can also be used to identify and determine pentachlorophenol. These methods must be preceded by purification steps which effectively separate the pentachlorophenol from interfering substances with similar absorptivity.

Chromatography has become very important as a method of separation and as a means of assay. High-pressure liquid chromatography with Amberlite XAD-7 resin (Fritz and Willis, 1973) has been used recently to separate complex mixtures of phenols. Thin-layer chromatography and paper chromatography can be used to separate pentachlorophenol from many interfering substances (Bevenue and Beckman, 1967). Thin-layer chromatography can also be used to estimate semiquantitatively the amount of the compound present in microgram quantities (Zigler and Phillips, 1967; Davies and Thuraisingham, 1968; Geike, 1972; and Frei-Häusler, Frei, and Hutzinger, 1973).

The most widely used technique for analysis of pentachlorophenol in practically all sample types is gas-liquid chromatography. The electron-capture detector is used routinely because of its high sensitivity to halogenated compounds; quantities in the nanogram to picogram range (10^{-9} to 10^{-12} g) can be measured. The gas-liquid chromatography technique is often sufficient to analyze and identify pentachlorophenol if retention times are determined on two or more columns.

When more rigorous identification is required and when a sufficient amount of sample is available for the collection of a gas chromatographic fraction of the suspected pentachlorophenol, final confirmation of identity must be obtained by ultraviolet or infrared spectroscopy. With the advent of computerized gas chromatography-mass spectrometer interfaces (Elkin et al., 1973), it is possible to scan automatically the mass fragments expected from the pentachlorophenol molecule. This technique uses the mass spectrometer as a molecule-specific detector. There are also less sophisticated systems in which the mass spectrometer is used to take the spectrum of only a few selected peaks. This technique is still very useful because the mass spectrum of a compound is normally an unambiguous identifier.

Pentachlorophenol is most often isolated from samples by a series of liquid-liquid extractions. The most common technique for soil samples is extraction with sodium or potassium hydroxide, followed by acidification of the extract and extraction from the acid solution with a nonpolar solvent such as benzene, toluene, or petroleum ether. The phenol is then reextracted from the nonpolar solvent with a basic aqueous solution if further purification is desired. Biological tissue samples have been treated in much the same way, although sometimes the tissue is treated with concentrated sulfuric acid (Erne, 1958) and anhydrous sodium sulfate and extracted by a Soxhlet technique. Recovery is variable (90% to 95%) but is similar to other methods. Steam distillation is used for isolation of larger (milligram) amounts of pentachlorophenol in soil and biological tissue. Water samples are easiest to handle. Most investigators simply acidify a large amount of water and extract it directly with a nonpolar organic solvent. Recoveries are greater (>95%) and detection limits lower (microgram/liter) for water samples than for other environmental samples (Stark, 1969; Rudling, 1970; Chau and Coburn, 1974; Renberg, 1974).

Some problems with isolation techniques have been reported. Difficulties were encountered in removal of pentachlorophenol from samples with a high fat content (Renberg, 1974), especially fat of marine origin, and from certain soil samples which formed gels when their alkaline extract was acidified to a pH lower than 6 (Stark, 1969). Because pentachlorophenol has a pK value of 5, extraction under these circumstances can be difficult or impossible. These difficulties can be eliminated by ion-exchange reactions (Renberg, 1974). In this procedure, acidic substances are bound to an anion exchanger and the liquid phase can be discharged. This method gave recovery values of >97% from soil and water and of 92% from fish.

In summary, the chemical methods for isolation and determination of pentachlorophenol exploit the dual nature of the compound — it exists as a polar anion under basic conditions and as a nonpolar molecule when acidified. This property allows its partitioning to be controlled easily.

SECTION D.2

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D.3 BIOLOGICAL ASPECTS IN MICROORGANISMS

D.3.1 BACTERIA

D.3.1.1 Metabolism

Bacterial metabolism and detoxification of pentachlorophenol occurs in the environment and in the laboratory. Because pentachlorophenol degradation and metabolism by microorganisms is an important facet of its dissipation from the environment, this subject is discussed extensively in Section D.7.3.5; only a brief overview is presented in this section.

Certain soil bacteria are able to detoxify pentachlorophenol by methylation, which results in the formation of pentachloroanisole (Suzuki and Nose, 1971, as cited in Cserjesi, 1972). Bacteria resistant to the toxic effect of sodium pentachlorophenate have been isolated from ponderosa pine stakes treated with pentachlorophenol (Morton, Stewart, and Bruneau, 1969). Bacteria isolated from treated and untreated stakes were capable of growing on media containing 0.5% pentachlorophenol in laboratory tests. The authors pointed out that the significance of this pentachlorophenol-resistant bacterium to the ultimate fate of the preservative is not known. The bacteria (not identified) may play a role in modification of pentachlorophenol, but this possibility has not been examined.

Chu and Kirsch (1972, 1973) isolated a bacterial strain (designated KC-3) from a continuous-flow enrichment culture shown to metabolize pentachlorophenol as a sole source of organic carbon and energy. Pentachlorophenol was degraded readily by the organism with the release of high levels of chloride, quantitative disappearance of the substrate, and an almost quantitative oxygen uptake. The source of the initial bacterial inoculation from which KC-3 was isolated was not described.

Kirsch and Etzel (1973) cultured an acclimated mixed bacterial population capable of biodegrading sodium pentachlorophenate; biodegradation was measured by release of ^{14}C during a 24-hr exposure. The initial inoculum was isolated from soil obtained from the grounds of a wood products manufacturer who used pentachlorophenol as a preservative. The initial sample was obtained from an area where the soil was well saturated with pentachlorophenol. The culture was gradually acclimated to increasing levels of pentachlorophenol until, after three months, pentachlorophenol was added to the cultures at a level of 120 mg/day. At this point the mixed culture (both proliferating and nonproliferating) was capable of pentachlorophenol biodegradation, but no attempt was made to characterize the species.

Watanabe (1973) isolated a bacterial culture capable of growing on and degrading pentachlorophenol in a medium containing organic salts and 40 mg/liter pentachlorophenol as a sole source of carbon. He determined from morphological and physiological properties that the bacteria was of the genus *Pseudomonas* or a closely related genus. The pathways of pentachlorophenol metabolism in bacteria have not been elucidated.

D.3.1.2 Effects

Pentachlorophenol and sodium pentachlorophenate are toxic or inhibitory to most forms of bacteria. In fact, pentachlorophenol is a well-known, widely used antimicrobial substance. Most industrial uses of pentachlorophenol depend on its ability to serve as a wood preservative by controlling bacteria and fungi. Detailed information on the mechanisms of pentachlorophenol toxicity to bacteria is scanty; however, the uncoupling of oxidative phosphorylation is generally believed to be of major importance. Many bacteria which are susceptible to control by pentachlorophenol depend primarily on anaerobic fermentation for their energy source. Thus, a mechanism for pentachlorophenol toxicity must include an explanation for the sensitivity of anaerobes to this compound. The antimicrobial efficiency of pentachlorophenol for various species of bacteria and fungi is presented in Table D.3.1. Growth responses of three other species of bacteria are shown in Table D.3.2.

TABLE D.3.1. ANTIMICROBIAL EFFICIENCIES OF PENTACHLOROPHENOL[a]

Test organism	Concentration of pentachlorophenol for inhibition (mg/liter)
Trichoderma viride	25-50
Trichoderma sp.	10-25
Ceratocystis pilifera	5-10
Polyporus tulipiferae	<1
Rhizopus stolonifer	1
Lenzites trabea	1-2.5
Ceratocystis ips	10-25
Chaetomium globosum	1-2.5
Aspergillus niger	10-25
Bacillus cereus var. *mycoides*	5-10
Bacillus subtilis	50-100
Escherichia coli	250-500
Pseudomonas aeruginosa	1000-2500
Enterobacter aerogenes	500-1000
Streptomyces griseus	5-10
Flavobacterium arborescens	2.5-5

[a] Dow Chemical Company purified grade, proprietary name Dowicide EC-7.

Source: Adapted from Dow Chemical Company, undated, p. 2. Reprinted by permission of the publisher.

TABLE D.1.2. GROWTH INHIBITION OF BACTERIA BY PENTACHLOROPHENOL

Species	Minimal response concentration of pentachlorophenol (μg/ml)	Inhibition (%)
[illegible]	10	[illegible]
[illegible]	25	65.8
[illegible] sp.	5	100

[a] Significant at the 5% level.
[b] Significant at the 1% level.

Source: Adapted from Breazeale and Camper, 1972, Table 1, p. 432. Reprinted by permission of the publisher.

Mickelson (1974) studied the inhibitory effect of pentachlorophenol on the growth of *Streptococcus agalactiae*. Aerobic molar growth yields (growth per mole of substrate) of the bacterium were depressed in the presence of pentachlorophenol to values equal to or less than those supported by substrate-level phosphorylation. The uncoupling action of pentachlorophenol was assumed to be responsible for this inhibitory effect. When the only source of energy available to the organism was from substrate-level phosphorylation (under conditions where anaerobic growth occurred), a severe depression of the molar growth yield was noted in the presence of pentachlorophenol. These results indicate that pentachlorophenol was also affecting the generation of ATP by substrate-level phosphorylation, but the mechanism of this process is not understood. Thus, in bacterial systems pentachlorophenol apparently affects not only oxidative phosphorylation, but also generation of ATP at the level of glycolysis or effective utilization of the ATP generated at this level. Inhibition of glycolytic enzymes, which has been demonstrated in higher animals, may be responsible for the effects noted in bacteria.

Heidman, Kincanron, and Gaudy (1967) studied the metabolic responses of activated sludge to the addition of sodium pentachlorophenate. Prolonged exposure resulted in significant changes in the predominant species of bacteria in the activated sludge, but the specific nature of these changes was not examined. Under conditions of shock loading, fairly small doses of sodium pentachlorophenate impaired the efficiency of the system. Successively more deleterious effects were seen in response to 5, 15, and 30 mg/liter sodium pentachlorophenate. Because concentrations required to inhibit bacterial cell growth vary from 1 to 2500 ppm pentachlorophenol for different species, it was not possible to determine whether the inhibition was due to an overall effect on all bacterial species or to an increasingly severe selection of species under shock-loading conditions. This interference with the degradative activities of sludge bacteria has important ramifications for biological treatment systems. Heidman, Kincannon, and Gaudy (1967) found that with sufficient acclimation an activated sludge system can operate in the presence of sodium pentachlorophenate concentrations as high as 250 mg/liter without serious retardation of biochemical purification efficiency. Apparently, the most serious threat to efficient activated sludge treatment of sewage by pentachlorophenol is under shock-loading conditions. The question of how pentachlorophenol affects the normal biological breakdown of glucose was also addressed in this study. No attempt was made to assess the degree of biological breakdown of pentachlorophenol in the activated sludge treatment. Sodium pentachlorophenate was not used as a substrate by the bacteria and did not exhibit any detectable biochemical oxygen demand.

D.3.2 FUNGI

D.3.2.1 Metabolism

Some species of fungi which tolerate the presence of pentachlorophenol are able to grow and reproduce in its presence, but they do not possess the ability to degrade the preservative. Other species of resistant fungi are able to degrade pentachlorophenol in treated wood and in soil block tests. For example, Banerjee (1967) demonstrated that an isolate of *Cephaloascus fragrans* was tolerant to concentrations of pentachlorophenol found in treated wood, but it did not have the capacity to degrade the preservative. On the other hand, three *Trichoderma* spp. were capable of tolerating the compound and degrading it in malt extract solutions. This degradation took place during a 12-day incubation period. Unligil (1968) isolated 27 strains of fungi — some from wood treated with fungicides — and determined the tolerance of pentachlorophenol on malt agar. The most tolerant fungi were *Trichoderma viride*, *Gliocladium viride*, and *Cephaloascus fragrans* (Table D.3.3). Species more sensitive than these three included *Alternaria dianthi*, *A. tenuis*, *Chaetomium globosum*, *C. indicum*, *Coniophora puteana*, *Daldinia concentrica*, *Graphium penicillioides*, *Hormodendrum cladosporioides*, *Hypoxylon* sp., *Lenzites saepiaria*, *Libertella* sp., *Phoma* sp., *Polystictus versicolor*, *Pullularia pullulans*, *Stereum hirsutum*, *Trametes variiformis*, and *Trogia crispa*. Within 52 days, *T. viride* and *C. puteana* depleted approximately 62% of the pentachlorophenol from samples with an initial content of 5.8 kg/m^3. Duncan and Deverall (1964) also demonstrated the ability of several common wood-inhabiting fungi to deplete pentachlorophenol in treated wood blocks. These species did not produce wood decay following degradation of the preservative.

The mechanism of pentachlorophenol detoxification by fungi is not well understood. Methylation of the compound has been suggested as a primary step in detoxification. Fungi have been shown to produce pentachloroanisole from pentachlorophenol during degradative processes (Curtis et al., 1974). This methylation reaction is thought to occur in boiler house litter, causing musty taint in chickens. It also seems likely that musty taint in water supplies may result from a similar process. A possible detoxification scheme for pentachlorophenol has been outlined

TABLE D.3.3. LENGTH OF LAG PHASE AND RATE OF GROWTH OF *TRICHODERMA VIRIDE*, *GLIOCLADIUM VIRIDE*, AND *CEPHALOASCUS FRAGRANS* IN MALT AGAR CONTAINING SODIUM PENTACHLOROPHENATE

Concentration of sodium pentachlorophenate (moles/liter)	*T. viride*		*G. viride*		*C. fragrans*	
	Lag phase (days)	Growth rate (mm/day)	Lag phase (days)	Growth rate (mm/day)	Lag phase (days)	Growth rate (mm/day)
0	0.6	35.7	0.6	17.9	0.6	1.7
1×10^{-6}	1.2	3.04	1.2	1.85	1.2	0.60
2×10^{-6}	2.2	1.65	2.0	0.94	2.0	0.31
4×10^{-6}	3.0	0.87	2.6	0.52	2.4	0.19
8×10^{-6}	5.4	0.48	No growth		No growth	

Source: Adapted from Unligil, 1968, Table 2, p. 48. Reprinted by permission of the publisher.

by Stranks (1976) (Figure D.3.1). According to this scheme, pentachlorophenol is oxidized to quinoids, chloranil, and tetrachlorobenzoquinone-(1,2). These compounds then combine with pentachlorophenol to yield trichloro-(pentachlorophenoxy)-benzoquinone-(1,4) or dichloro-bis(pentachlorophenoxy)-benzoquinone-(1,2).

D.3.2.2 Effects

Most data on the toxicity of pentachlorophenol to fungi deal with common wood-destroying fungi (Table D.3.4). Interest in the antifungal activity of pentachlorophenol stems from attempts to determine the most efficient use of the preservative for control of fungi in treated wood. Table D.3.5 lists the toxic effects of pentachlorophenol on a variety of commonly found fungi. The effect of pentachlorophenol on spore germination, growth, and sporulation of three fungal plant pathogens is shown in Table D.3.6. The minimum effective concentration of pentachlorophenol for inhibition of radial growth of *Trichoderma viride* was 1.2×10^{-6} moles/liter (Blackman, Parke, and Garton, 1955).

D.3.3 ALGAE

D.3.3.1 Metabolism

No information was found on the metabolism of pentachlorophenol by algae.

D.3.3.2 Effects

Pentachlorophenol is extremely toxic to algae. Huang and Gloyna (1967) reported that pentachlorophenol was the most toxic compound among ten halogenated phenols tested on *Chlorella pyrenoidosa*. The lethal dosage of pentachlorophenol was 7.5 μg/liter. Even at a concentration as low as 1.5 μg/liter, pentachlorophenol inhibited chlorophyll synthesis for two days.

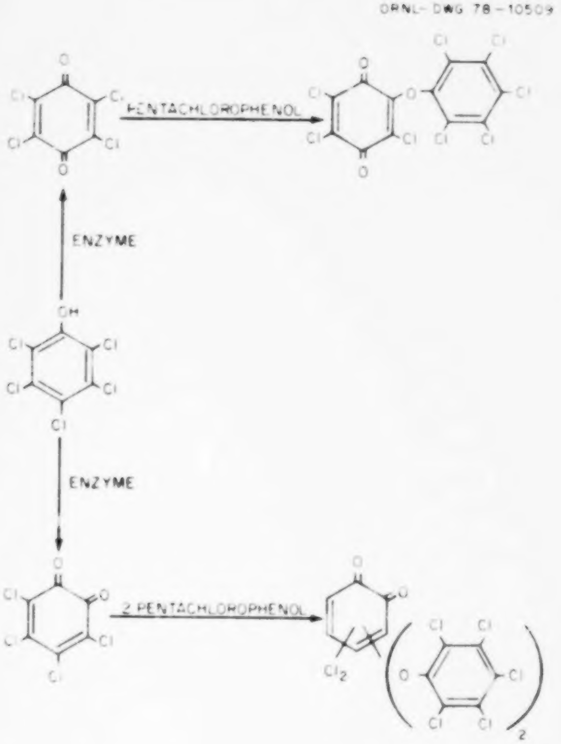

Figure D.3.1. Probable detoxification of pentachlorophenol by the laccase of *Coriolus versicolor*. Source: Adapted from Stranks, 1976, Figure 2, p. 14. Reproduced by permission of the Minister of Supply and Services Canada.

Pentachlorophenol and sodium pentachlorophenate were effectively used as algicides for the control of stoneworts of the genera *Chara* and *Nitella* in paddy fields in West Bengal (Table D.3.7) (Mukherji, 1972). At doses as low as 3.75 kg/ha, small snails and fish were killed by the compounds. Some minor damage to the rice crop occurred as small spots which appeared on the young leaves, but no adverse effects on crop growth were noted. A 2.0 mg/liter solution of sodium pentachlorophenate and sodium salts of other phenols in the culture medium inhibited 50% of the test algae in a 21-day laboratory incubation test (Palmer and Maloney, 1955). The growth of algae in filtered water from a spray pond used for storing cooling water was prevented by 15 mg/liter sodium pentachlorophenate; in pond water containing algae, growth was stopped in seven days (Gelfland, 1941). Algae stopped growing immediately in water containing a concentration of 20 mg/liter sodium pentachlorophenate.

TABLE D.3.4. THRESHOLD VALUES FOR PENTACHLOROPHENOL TOXICITY TO WOOD-DESTROYING FUNGI

	Test fungus	Threshold pentachlorophenol	
		(kg/m^3)	(lb/ft^3)
I.	*Fomes subroseus*	3.85	0.24
	Polystictus tulipferae	1.77	0.11
II.	*Poria incrassata*	1.2	0.075
	Poria monticola B	0.98	0.061
	Polystictus adustus	0.98	0.061
	Poria monticola A	0.85	0.053
	Polystictus versicolor	0.77	0.048
	Polystictus hirsutus	0.69	0.043
III.	*Lenzites trabea* A	0.63	0.039
	Lentinus lepideus	0.48	0.030
	Polystictus abietinus	0.48	0.030
	Poria vaillantii	0.48	0.030
	Poria xantha	0.40	0.025
	Lenzites trabea B	0.35	0.022
	Daedalea quercina	0.35	0.022
	Lenzites saepiaria	0.18	0.011
	Schizophyllum commune	0.18	0.011
	Stereum frustulosum	0.18	0.011

Source: Adapted from Sproule, 1960, p. 46. Reprinted by permission of the publisher.

TABLE D.3.5. TOXICITY OF PENTACHLOROPHENOL AND SODIUM PENTACHLOROPHENATE TO FUNGI

Fungus	Amount causing total inhibition (mg/liter)		Amount causing death (mg/liter)	
	Pentachlorophenol	Sodium pentachlorophenate	Pentachlorophenol	Sodium pentachlorophenate
Ceratostomella pilifera	60	60	60	80
Hormonema dematioides	60	60	60	80
Hormodendrum cladosporioides	60	500	500	300
Polystictus versicolor	80	100	100	100
Polystictus hirsutus	80	200	200	100
Coniophora cerebella	20	20	20	20
Fomes roseus	60	60	60	60
F.P.L. No. 517[a]	20	30	60	60
Lentinus lepideus	20	20	40	40
Poria incrassata	20	20	20	20
Trametes serialis	10	20	20	20
Lenzites saepiaria	20	20	60	40
Lenzites trabea	20	20	40	40
Merulius [illegible]	<10	<10	<10	<10
U-10	20	20	20	20
Trichophyton rosaceum	40	40	60	40
Trichophyton interdigitale	60	60	80	80
Epidermophyton inguinale	40	40	40	60
Aspergillus niger	60	60		
Penicillium chrysogenum	60	60	80	60
Penicillium digitatum	40	40	40	60
Alternaria radicina	60	60	60	60
Rhizopus nigricans	40	80	60	80
Fusarium vasinfectum	100	100	200	200

[a] Formerly known as *Fomes annosus*.

Source: Adapted from Carswell and Nason, 1938, Table II, p. 625. Reprinted by permission of the publisher.

TABLE D.3.6. EFFECTS OF PENTACHLOROPHENOL (0.01 mole/liter) ON SPORE GERMINATION, GROWTH, AND SPORULATION OF *HELMINTHOSPORIUM ORYZAE*, *ALTERNARIA SOLANI*, AND *CURVULARIA LUNATA*

Compound	*H. oryzae*			*A. solani*			*C. lunata*		
	Germination (%)	Growth[a]	Sporulation[b]	Germination (%)	Growth[a]	Sporulation[b]	Germination (%)	Growth[a]	Sporulation[b]
Pentachlorophenol	0	1	0	0	1	1	0	1	0
Control	80	3	3	85	3	3	50	3	3
Low pH control (malic acid)	80	3	3	84	3	3	50	3	3

[a] 0, 1, 2, and 3 indicate nil, low, moderate, and high growth.
[b] 0, 1, 2, and 3 indicate nil, low, moderate, and high sporulation.

Source: Adapted from Mukherjee and Kundu, 1973, Table 1, p. 90. Reprinted by permission of the publisher.

TABLE D.3.7. CONTROL OF *CHARA* AND *NITELLA* SPECIES WITH CHEMICALS APPLIED 60 DAYS AFTER TRANSPLANTING THE PADDY

Location of application	Days after application	Pentachlorophenol		Sodium pentachlorophenate	
		Dose (kg/ha)	Algae killed (%)	Dose (kg/ha)	Algae killed (%)
Ramchandrapur, West Bengal, 1970	15	5.0	90	5.0	95
Siddheswarpur, West Bengal, 1971	7	3.75	90	2.5	75
	16	3.75	95	2.5	90
Janerdanpur, West Bengal, 1971	7	4.4	90	3.75	100
	16	4.4	100	3.75	100

Source: Adapted from Mukherji, 1972, Table 1, p. 390. Reprinted by permission of the publisher.

SECTION D.3

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D.4 BIOLOGICAL ASPECTS IN PLANTS

D.4.1 METABOLISM

Very little information is available on the distribution and transport of pentachlorophenol in vascular plants. Hilton, Yuen, and Nomura (1970) studied the distribution of pentachlorophenol residues in sugar cane following either foliar or root application. Both sides of the three top leaves were painted with [^{14}C]pentachlorophenol in an amount equivalent to 6 mg of sodium pentachlorophenate per plant in aqueous solution containing 10% methanol. Samples taken at two, four, and eight weeks included treated leaves, untreated green leaves, dry leaf trash, stalks, roots, and suckers. These samples were analyzed for ^{14}C and, in some cases, the identity of the labeled components was verified by electron-capture gas chromatography. Between the first and last sampling period about 16% of the ^{14}C[pentachlorophenol] was lost from the plants. This radioactivity did not appear in any of the plant samples or in the nutrient solution in which the plants were grown; thus, it was presumed to be lost from the treated leaves by vaporization or weathering. Table D.4.1 shows that the radioactivity which remained was confined primarily to the treated leaves. Small amounts of pentachlorophenol were apparently distributed to all parts of the plant except the roots. The authors suggested that the residual radioactivity found in other portions of the plant may have resulted from translocation of the vapor or from mechanical transfer of pentachlorophenol to untreated portions of the plant. At eight weeks the leaves treated with pentachlorophenol were the lowest green leaves on the plants. Following abscission of the leaves (about one leaf per ten days), the radioactivity was lost from

TABLE D.4.1. DISTRIBUTION OF [^{14}C]PENTACHLOROPHENOL IN SUGAR CANE AFTER FOLIAR APPLICATION

Plant part	Distribution of recoverable radioactivity (%)		
	2 weeks after application	4 weeks after application	8 weeks after application
Treated leaves	100	82	84
Untreated green leaves	0	11	6.6
Untreated, dry leaf trash	0	2.7	2.0
Stalk	0	4.6	7.1
Roots	0	0	0
Suckers	0	0	0.7

Source: Adapted from Hilton, Yuen, and Nomura, Table I, p. 218. Reprinted by permission of the publisher.

the plant. The amount of pentachlorophenol applied to these plants was large enough to cause toxic symptoms such as moderate root and leaf injury.

Hilton, Yuen, and Nomura (1970) also investigated pentachlorophenol distribution after root application. Sugar cane plants were grown in covered crocks containing 3 liters of aerated nutrient solution with an initial pentachlorophenol level of 5 mg/liter. The plants remained in the radioactive solution for four weeks and then were removed; the roots were washed, and the plants were placed in fresh nutrient solution free of pentachlorophenol. Parts of the plant were removed at zero, four, and eight weeks following transfer, and the samples were prepared and analyzed in a manner similar to the methods used for samples from plants subjected to foliar treatment. During the four-week treatment period, the radioactivity of the nutrient solution decreased 86%. Aerated solutions without plants showed extensive volatilization of pentachlorophenol. However, considerable amounts of pentachlorophenol were taken up by the roots; radioactivity decreased rapidly and exponentially in the nutrient solutions. Of the 15 mg of pentachlorophenol applied, roughly 13.5 mg was recovered from the plants at the end of the four-week exposure. During the eight-week period when plants were growing in fresh nutrient solutions, the pentachlorophenol content of the plants decreased by about 60%. More than 99% of the radioactivity absorbed and retained was found in the root system; some remaining activity appeared in the stalk and suckers (Table D.4.2). Pentachlorophenol was apparently retained in the root system throughout the

TABLE D.4.2. DISTRIBUTION OF [^{14}C]PENTACHLOROPHENOL IN SUGAR CANE GROWN FOR FOUR WEEKS IN PENTACHLOROPHENOL-TREATED NUTRIENT SOLUTION AND TRANSFERRED TO PENTACHLOROPHENOL-FREE SOLUTION

Plant part	Distribution of recoverable radioactivity (%)		
	0 weeks	4 weeks after transfer	8 weeks after transfer
Dry leaf trash	0	0	0
Green leaves	0	0	0
Stalk	0.8	3.1	4.6
Roots	99	97	94
Suckers			1.6

Source: Adapted from Hilton, Yuen, and Nomura, 1970, Table II, p. 219. Reprinted by permission of the publisher.

entire experiment. Losses of pentachlorophenol from the root system occurred without a concomitant increase in the pentachlorophenol content of other plant parts or of the nutrient solution. The authors suggested that the gradual release of radioactivity from the root system was followed by volatilization of pentachlorophenol from the nutrient solution. Electron-capture gas chromatography of root extracts indicated that <10% of the residue had a retention time identical to that of pentachlorophenol. Hilton, Yuen, and Nomura (1970) stated that "the remainder of the chromatographed extract exhibited two small peaks with longer retention time than any of the known chlorinated phenols." The substances, presumed to be metabolic breakdown products of pentachlorophenol, were not identified. At the same time these investigators also studied the translocation and distribution of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and ametryne (2-ethylamino-4-isopropylamino-6-methylthio-*s*-triazine) and provided substantial evidence that these pesticides are translocated readily from roots to leaves. Under identical test conditions, pentachlorophenol apparently does not translocate readily but remains at the site of treatment until the substance is metabolized or released from the plant tissue.

Conversely, Miller and Aboul-Ela (1969) found evidence of pentachlorophenol translocation in cotton plants (Deltapine variety) grown to maturity in a greenhouse. Plants sprayed with [^{14}C]pentachlorophenol accumulated up to 2 μg/g pentachlorophenol (or its metabolites) in the cotton seed kernels of bolls which were closed at the time of spraying; no pentachlorophenol was detected, however, in kernels from open bolls. Thus, pentachlorophenol was translocated from the external area of the bolls to the kernels. The distribution of pentachlorophenol applied in single droplets of diesel oil to young cotton seedlings was also determined. In separate experiments, pentachlorophenol was applied either to the point of veinal anastomosis of the first true leaf or at the juncture of the cotyledon and its petiole. Application to the first true leaf resulted in some absorption and translocation through the veins of the leaf within 1 hr. Pentachlorophenol was evenly distributed through the veins of the treated leaf after 8 hr, but no movement out of the treated leaf was observed after eight days. When pentachlorophenol was applied to the cotyledon, the compound was distributed uniformly through the veins of the cotyledonary petiole within 4 hr. One day after pentachlorophenol application, all parts of the seedling except the other cotyledon and the roots contained detectable levels of pentachlorophenol. Apparently, radioactivity tends to concentrate in the glandular hairs. Regrowth leaves from defoliated mature cotton plants contained an average of 1.03 μg/g pentachlorophenol.

D.4.2 EFFECTS

D.4.2.1 Physiological or Biochemical Role

No evidence suggests that pentachlorophenol plays any normal role in the physiology of plants; thus, no nutritional requirement exists.

D.4.2.2 Toxicity

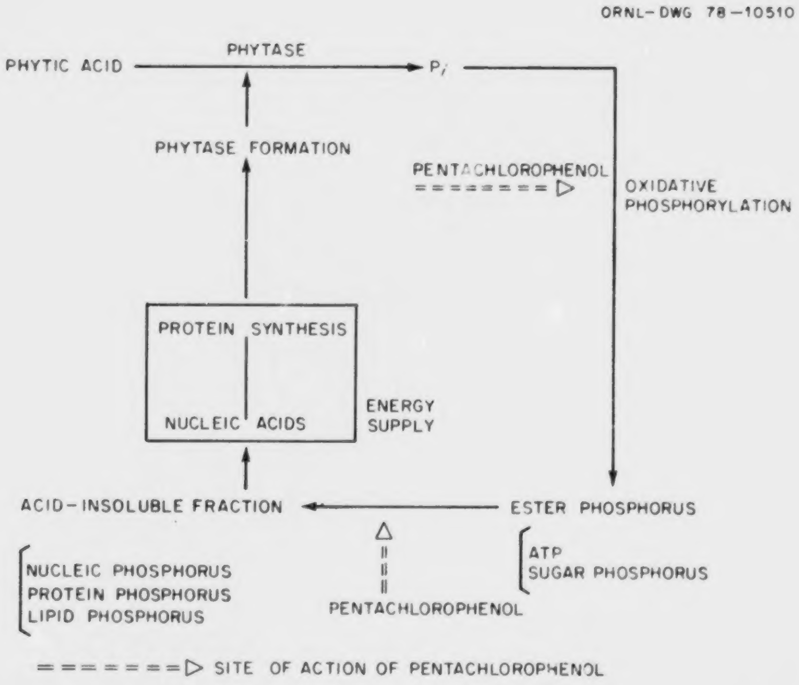
D.4.2.2.1 Mechanism of Action — Weinbach (1957) demonstrated that pentachlorophenol can uncouple oxidative phosphorylation in isolated animal mitochondria. Similarly, experiments by Uesugi and Fukunaga (1967, as cited by Takahashi, 1971) with the mycelia of *Piricularia oryzae*, a pathogen of rice blast, and by Matsunaka (1965, as cited by Takahashi, 1971) with cauliflower mitochondria showed that 10^{-6} mole of pentachlorophenol per liter produced an uncoupling action in plants. These studies indicate that the uncoupling of oxidative phosphorylation is likely the primary mechanism of pentachlorophenol phytotoxicity. The mechanisms of the action of pentachlorophenol on plants have not been described in detail; further investigations are warranted.

Takahashi (1971) studied the mechanism of suppression of germination by pentachlorophenol based on phytate decomposition in germinating rice seeds. Phosphorus appears in seeds primarily in organic form, and as much as 80% of the total phosphorus may be in the form of phytic acid. The level of inorganic phosphate may be the limiting factor in phosphate-requiring metabolic reactions in the germinating seed. The large amount of phytate present in seeds may serve as a source of inorganic phosphate. Thus, liberation of phosphorus by decomposition of phytate may be essential for germination to take place. By pretreating rice seeds with pentachlorophenol, Takahashi (1971) found that pentachlorophenol caused continuous decomposition of "ester-phosphorus" in the germinating seedlings. The author proposed that this effect of pentachlorophenol together with its effect of uncoupling oxidative phosphorylation could be responsible for the suppression of germination caused by insufficient usable energy. The insufficient energy supply results in inadequate synthesis of phytase, thereby short-circuiting the normal release of inorganic phosphate from phytic acid. This proposed metabolic scheme and the points of action of pentachlorophenol in the energy pathway of the developing seed are shown in Figure D.4.1.

D.4.2.2.2 General Toxicity

D.4.2.2.2.1 Terrestrial plants — Pentachlorophenol is generally toxic to vascular plants. Pentachlorophenol-treated flats used to grow seedlings were tested for possible injurious effects on transplanted tomato plants (Kaufert and Loerch, 1955). The flats were treated with 5% pentachlorophenol either in Stoddard solvent or in No. 2 fuel oil and were allowed to stand in a heated room for one month to eliminate most of the solvent. The tomato plants were severely damaged in both instances. Furthermore, plants started in these flats in subsequent years were also damaged (Table D.4.3).

The effect of using pentachlorophenol-treated wooden trays to rear coniferous seedlings for reforestation was studied by Ferguson (1959). The containers were treated with 5% pentachlorophenol in diesel oil in a 4-hr cold-soak process, after which they were allowed to weather for 30 days prior to the planting of seedlings. All species tested were severely damaged except longleaf pine (*Pinus palustris* Mill.). Symptoms included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PHYTIC ACID
PHYTASE
P_i
PHYTASE FORMATION
PENTACHLOROPHENOL
OXIDATIVE PHOSPHORYLATION
PROTEIN SYNTHESIS
NUCLEIC ACIDS
ENERGY SUPPLY
ACID-INSOLUBLE FRACTION
ESTER PHOSPHORUS
NUCLEIC PHOSPHORUS
PROTEIN PHOSPHORUS
LIPID PHOSPHORUS
PENTACHLOROPHENOL
ATP
SUGAR PHOSPHORUS
SITE OF ACTION OF PENTACHLOROPHENOL

Figure D.4.1. Schematic illustration of the inhibition of phytate decomposition by pentachlorophenol in germinating rice seeds. Source: Adapted from Takahashi, 1971, Figure 13, p. 23. Reprinted by permission of the publisher.

TABLE D.4.3. EFFECT OF PENTACHLOROPHENOL PRESERVATIVE TREATMENT OF WOODEN FLATS ON THE GROWTH OF TOMATO PLANTS

Treatment	Retention (kg/m³)	Extent of injury to tomato plants			
		1st year	2nd year	3rd year	4th year
5% pentachlorophenol in Stoddard solvent	11	Severe	Light	None	None
5% pentachlorophenol in No. 2 fuel oil	8	Severe	Moderate	Light	None
Untreated sap pine		None	None	None	None
Cypress heartwood		None	None	None	None

Source: Adapted from Kaufert and Loerch, 1955, p. 2. Reprinted by permission of the publisher.

needle twisting, followed by blanching and desiccation (Table D.4.4). Plants grown in untreated flats which were adjacent to and west of pentachlorophenol-treated flats were also damaged. The greenhouse was cooled by exhaust fans in the east wall, and the prevailing air movement was from east to west; therefore, it was suggested that damage was caused by volatile compounds transported in air. This premise was further supported by experiments in which soil from the pentachlorophenol-treated flats was interchanged with soil from untreated flats. Mortality and damage occurred only in the flats originally treated with pentachlorophenol. For the second crop, although the damage seen in these flats was less than 50% of that in the first crop, the persistence of pentachlorophenol was still substantial. Table D.4.4 shows that different coniferous species vary considerably in their reactions to pentachlorophenol. Slash pine (*Pinus caribaea* Morel.), loblolly pine (*P. taeda* L.), and shortleaf pine (*P. echinata* Mill.) suffered extensive mortality, but longleaf pine (*P. palustris* Mill.) suffered no mortality, although it displayed some symptoms of injury.

TABLE D.4.4. DEATH OR INJURY TO CONIFER SEEDLINGS GROWN IN PENTACHLOROPHENOL-TREATED WOODEN TRAYS

Pine species	Diesel oil		5% pentachlorophenol in diesel oil	
	Killed (%)	Injured (%)	Killed (%)	Injured (%)
Pinus caribaea	0	4	86	8
Pinus taeda	0	6	94	5
Pinus echinata	0	9	90	9
Pinus palustris	0	0	0	50

Source: Adapted from Ferguson, 1959, p. 22.

Carlson and Nairn (1974-1975) tested the effect of pentachlorophenol-treated wood on containerized red pine (*P. resinosa* Ait.) and jack pine (*P. banksiana* Lamb.) seedlings. Severe injuries developed in both species. Symptoms occurred within two days after initial exposure to pentachlorophenol fumes. The flats used were treated with a 5% pentachlorophenol preservative and then dried in a fume hood for three weeks prior to use. Seedlings exposed to pentachlorophenol developed more slowly and exhibited necrosis and twisted needles. Twisting of the needles became more pronounced, and eventually the seedlings dropped over at the base and died. The sequence of events was very similar for both species; however, red pine was slightly more resistant (Table D.4.5).

TABLE D.4.5. DEATH OR INJURY TO RED AND JACK PINE SEEDLINGS GROWN IN PENTACHLOROPHENOL-TREATED FLATS[a]

Pine species	No treatment[b]		Pentachlorophenol treatment[b]	
	Killed (%)	Curled (%)	Killed (%)	Curled (%)
Pinus banksiana	0 *a*	4.0 *b*	84 *c*	1.5 *b*
Pinus resinosa	0 *d*	1.3 *e*	78 *f*	2.4 *e*

[a] Number of seedlings observed ranged from 517 to 702.

[b] The small letters indicate Duncan's multiple range grouping of treatments which do not differ significantly at the 5% level.

Source: Adapted from Carlson and Nairn, 1974-1975, Tables 1 and 2, p. 34.

Ferree (1974) reported moderate to severe damage in high-density apple orchards where posts freshly treated with pentachlorophenol were used as supports for tree growth. Posts were set two to four weeks prior to planting of the trees. Within several months, leaf chlorosis was evident in all trees planted closest to the posts. Golden Delicious trees (*Malus domestica*) appeared to be the most susceptible to pentachlorophenol poisoning. Damage included bark lesions, chlorosis, and, in severe cases, death of the branch back to the graft union. Four trees died, and stunting and growth reduction were evident among those less severely affected. Ferree (1974) suggested hat if pentachlorophenol-treated posts are used, they should be weathered by outside exposure for one year.

In the past, pentachlorophenol formulations have been used extensively as herbicides in the United States and abroad. Although its use has declined in the United States, its use elsewhere has continued. Pentachlorophenol is used primarily as either a preemergence herbicide or as a directed spray in the removal of weeds from specific areas. Although different plant species have different degrees of susceptibility, pentachlorophenol cannot be considered a herbicide which possesses substantial specificity toward target species. Thus, when spraying is required to remove target species growing among crops, more effective alternatives for chemical weed control are available. Also, pentachlorophenol is used as a defoliant and preharvest desiccant. In tests by Bovey (1969), pentachlorophenol was the fastest-acting desiccant among six contact herbicides.

Pentachlorophenol applied at concentrations of 2 to 4 kg/ha caused considerable malformation in crested wheatgrass seedlings (Klomp and Hull, 1968). Outdoor test plots were sprayed with pentachlorophenol, and samples of the soil were removed at an unspecified time. These soil samples were placed in plastic pots in a greenhouse and were seeded with crested wheatgrass. Although yields were not reduced substantially, the grass displayed epinasty, onion leaf, and other malformations.

D.4.2.2.2.2 Aquatic plants — Blackman et al. (1955) determined the effect of pentachlorophenol on duckweed (*Lemna minor*). The index of toxicity used was the concentration of pentachlorophenol which caused chlorosis in half the fronds. This value was 1.2 x 10 micromoles/liter or 0.32 mg/liter.

Dharukar (1973) studied the effect of pentachlorophenol on water hyacinth (*Eichhornia crassipes*). Spraying ponds with sodium pentachlorophenate at a rate of 11.4 g/m^2 effectively controlled water hyacinth. Plants were sprayed in April, September, and December, and two months after spraying 92%, 43%, and 78%, respectively, had died. Because application was made on an area basis, the pentachlorophenol concentration could not be computed. Reinfestation occurred, but all plants surviving the treatment were stunted in growth and the formation of offsets was decreased.

D.4.2.2.3 Mitotic Effects — The effect of pentachlorophenol on root tip mitosis and germination of pollen grains in water hyacinth was also investigated by Dharurkar (1972). When root tips were cultured in solutions of sodium pentachlorophenate, the percentage of cells undergoing mitosis gradually decreased. The effect was dose related; 24 hr after treatment with 250 mg/liter pentachlorophenol only 2% of the cells showed mitotic activity. Chromosomal abnormalities such as stickiness of chromosomes, fragmentation, abnormal shapes of nuclei, grouping of chromosomes, multinucleolation, and bridges were observed. Also, the percentage of germinating pollen grains and the length of pollen tubes decreased as the concentration of pentachlorophenol increased. No germination occurred when plants were cultured at 6 mg/liter sodium pentachlorophenate.

Pentachlorophenol has caused abnormal mitosis in the European broad bean (*Vicia faba*). Amer and Ali (1969) demonstrated a positive effect of pentachlorophenol on the number of mitotic anomalies as well as a considerable decrease in the mitotic index of root preparations of *Vicia faba* (Table D.4.6). The degree of abnormality appeared to be dose related; of the cells examined, 6%, 4.3%, and 2.3% were found to be abnormal at dosages of 174, 87, and 43.5 mg/liter pentachlorophenol respectively.

D.4.2.2.4 Effect on Lipid Synthesis — Mann and Pu (1968) found an effect of pentachlorophenol on lipid synthesis in the excised hypocotyls of hemp sesbania [*Sesbania exaltata* (Raf.) Cory]. Concentrations of 1 to 20 mg/liter of pentachlorophenol were used, and the incorporation of [C-14]malonate into lipids was monitored. A dose-related inhibition of lipid synthesis by pentachlorophenol was noted; synthesis was 53% of normal at 20 mg/liter pentachlorophenol. No inhibition mechanisms were suggested.

TABLE D.4.6. EFFECT OF PENTACHLOROPHENOL ON MITOTIC ABNORMALITIES IN ROOT PREPARATIONS OF *VICIA FABA*

Cell type and mitotic stage	Control	43.5 mg/liter pentachlorophenol	87 mg/liter pentachlorophenol	174 mg/liter pentachlorophenol
Number of dividing cells	669	888	1109	522
Number of abnormal cells	10	20	48	31
Abnormal cells, %[a]	1.52	2.33	4.33	6.00
Cells in prophase, %	62.0	56.8	55.2	48.1
Cells in metaphase, %	19.9	14.2	22.8	29.5
Abnormal cells in metaphase, %[a]	4.51	7.14	10.2	12.3
Cells in anatelophase, %	18.1	29.1	22.0	22.4
Abnormal cells in anatelophase, %[a]	3.30	4.26	9.02	10.3
Mitotic index	98	77	61	39

[a] Determined as ratio of abnormal cells to normal cells x 100.

Source: Adapted from Amer and Ali, 1969, Table 1, p. 534. Reprinted by permission of the publisher.

SECTION D.4

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D.5 BIOLOGICAL ASPECTS IN WILD AND DOMESTIC ANIMALS

D.5.1 BIOLOGICAL ASPECTS IN BIRDS AND MAMMALS

D.5.1.1 Metabolism

D.5.1.1.1 Uptake and Absorption

D.5.1.1.1.1 Inhalation — Toxic responses in animals following the inhalation of pentachlorophenol have been reported by Plakhova (1966). Animals (species not stated) daily exposed for 4 hr to 23 mg pentachlorophenol per cubic meter of air for a period of four months showed decreases in hemoglobin, weight, and erythrocytes with accompanying increases in eosinophils and leucocytes. Exposure to a lower concentration of 7.44 mg/m^3 resulted in unstable, insignificant changes.

D.5.1.1.1.2 Cutaneous absorption — Walters (1952) studied the effects of pentachlorophenol-treated wood on pigs and cattle. In two separate experiments, pigs were confined to farrowing houses treated with a 5% solution of pentachlorophenol in mineral spirits, a light solvent that evaporates rapidly to leave a dry surface. In the first experiment, a sow and two-week-old litters of 6 to 12 pigs were confined to farrowing houses that had been treated with pentachlorophenol on the outside only. These animals remained in the farrowing houses until the young pigs were eight weeks old. The pigs displayed normal growth and showed no apparent detrimental effects from the exposure. No tests were performed to determine if pentachlorophenol was absorbed internally by the pigs. In the second experiment, the sows were allowed to farrow in houses treated inside and outside with pentachlorophenol. The treated houses were allowed to air-dry for 30 days before the sows were placed in the buildings. The sows and their litters were confined to the farrowing houses for at least 7 days and no ill effects were noted. Similarly, cattle exposed to pentachlorophenol-treated posts, gates, electric fence stakes, and corral timbers for up to eight months were unaffected. The timbers were soaked in a 5% solution of pentachlorophenol in No. 3 fuel oil. These experiments do not rule out cutaneous absorption of pentachlorophenol, but they do demonstrate that acutely or chronically toxic amounts of pentachlorophenol were not absorbed by the cutaneous route.

In contrast to the data of Walters (1952), Schipper (1961) provided evidence that pentachlorophenol can be cutaneously absorbed in acutely toxic amounts. Extensive losses of baby pigs confined to farrowing pens treated with pentachlorophenol occurred in North Dakota. The lumber was treated with a formulation containing 4.37% pentachlorophenol, 0.63% other chlorophenols, 81.6% petroleum distillate, and approximately 13.2% inert ingredients. The wood was allowed to dry for one to five days before it was used in the experiment. Direct skin contact by pregnant swine with farrowing pens freshly treated with pentachlorophenol solutions produced a toxic response which resulted in extensive mortality in newborn swine. Insufficiently dried lumber saturated with pentachlorophenol was most toxic. Direct contact of the pregnant sow with the freshly treated lumber apparently allowed sufficient skin absorption to cause extensive fetal mortality and the birth of weak pigs. Furthermore, the presence of pentachlorophenol on the teats and in the mammary glands apparently discouraged nursing. The piglets were probably exposed to pentachlorophenol by three routes — inhalation of vapors, oral uptake from the teats, and cutaneous contact with the treated wood. Autopsy of the piglets revealed pathological lesions of the kidney, urinary bladder, liver, spleen, stomach, and intestinal and respiratory tracts. In general, mortality at birth was greatest for pigs born from sows confined to the pentachlorophenol-treated pens for the longest time prior to farrowing (Table D.5.1). The degree of toxicosis in young pigs depended on their age at time of exposure (i.e., the older the pigs, the lower the toxic effect). The extreme sensitivity at very early ages probably resulted from an inability to excrete pentachlorophenol rapidly enough to prevent systemic toxicity. The inclusion of bedding in the pentachlorophenol-treated farrowing pens prevented the toxic response. The pentachlorophenol levels in the swine were not estimated.

TABLE D.5.1. MORTALITY OF PIGS FARROWED IN CRATES TREATED WITH PENTACHLOROPHENOL

Treatment[a]	Number of litters investigated	Number of pigs in litters	Number dead on delivery	Postfarrowing deaths[b]
Dilute pentachlorophenol	8	89	5	1/10, 2/4, 3/1
Dilute pentachlorophenol (factory-treated lumber)	3	33	5	1/4, 3/4, 4/1, 6/1
Undiluted pentachlorophenol	2	0	[c]	
Dilute pentachlorophenol (factory-treated lumber covered with straw)	2	19	0	1/1
Undiluted creosote	4	41	24	1/6, 2/4, 3/1
Distillate	1	11	0	None
Controls	4	36	1	1/1, 2/2

[a] Applied by brush unless otherwise described.

[b] Numerator is number of days following delivery; denominator is number of pigs that died on that day.

[c] Both sows died before delivery.

Source: Adapted from Schipper, 1961, Table 1, p. 403. Reprinted by permission of the publisher.

Another case of pentachlorophenol poisoning of newborn swine was reported by Blevins (1965). Nine newborn pigs exposed to a newly constructed farrowing house which was improperly treated with pentachlorophenol died. The owners exceeded the manufacturer's recommendation in treating the floor of the farrowing house with a solution of pentachlorophenol in crankcase oil, and no straw or shavings were provided as bedding. The author theorized "that these pigs received triple exposure to the wood preservative: 1) by direct absorption through the skin; 2) orally from the gilt's mammary glands, and 3) perhaps most severely, as an aerosol in the hot, poorly ventilated farrowing house."

The effect of pentachlorophenol-creosote mixtures on the clipped skin of calves was determined by Olafson and Leutritz (1959). Moderate to severe local damage was evident as a dry, crusty thickening of the skin seven to ten days following application. Healing was slow and recovery was incomplete even after four weeks; possible systemic effects were not determined.

D.5.1.1.1.3 Ingestion — Pentachlorophenol is repellent to animals. Pasture treated with a herbicidal formulation of pentachlorophenol was avoided by cattle (Grigsby and Farwell, 1950). The herbicide (containing 3.7 kg of pentachlorophenol in 106 liters of diesel fuel and emulsified with 76 liters of water) was applied to an acre of pastureland, and livestock, including horses, dairy and beef cattle, sheep, and swine, were tested for preference of treated or untreated areas. These animals avoided the sprayed areas. Pentachlorophenol was applied at a rate two to four times greater than the recommended herbicidal dosage. The authors concluded that agricultural use of these materials for weed control in pastures is a reasonably safe procedure. Despite the repellent characteristics, cattle will drink pentachlorophenol solutions when they are thirsty. Spencer (1957) reported fatal poisoning of a cow which drank an undetermined amount of 5% pentachlorophenol in kerosene. He noted that both kerosene and pentachlorophenol are toxic and that pentachlorophenol is a tissue fixative that prevents fermentation in the rumen.

Walters (1952) "drenched" (force-fed) swine with 35 g of a solution containing 5% pentachlorophenol, 5% diacetone alcohol, and 90% mineral spirits. The dose rate was approximately 83 mg/kg, and the blood level 48 hr after dosing was 42 mg/liter. No apparent harmful effects occurred in the swine; however, absorption across the gastrointestinal tract was shown by analysis of several tissues during autopsy. The retention and excretion of pentachlorophenol by these swine are shown in Table D.5.2.

Blood levels of pentachlorophenol in sheep force-fed pentachlorophenol-impregnated sawdust are shown in Table D.5.3 (Harrison, 1959). The rapid initial rise in the pentachlorophenol concentration in the blood indicated rapid absorption by the gut. Levels of pentachlorophenol in the blood of animals that survived peaked in 3 to 6 hr following ingestion. Wide variability is evident in both the blood levels reported and in the animals' responses to a pentachlorophenol dose. One sheep administered a dose of 139 mg/kg died after 12 hr, but another receiving an identical dose survived for 24 days, with pentachlorophenol levels in the blood dropping to near zero.

D.5.1.1.2 Transport and Distribution — The transport and distribution of pentachlorophenol in experimental animals is discussed in detail in Section D.6.1.2. It is assumed that data obtained from experimental animals are directly applicable to domestic and wild animals, particularly

TABLE D.5.2. RETENTION OF PENTACHLOROPHENOL IN VARIOUS BIOLOGICAL MATERIALS FROM SWINE DRENCHED WITH A SOLUTION CONTAINING 5% PENTACHLOROPHENOL, 5% DIACETONE ALCOHOL, AND 90% MINERAL SPIRITS

Biological material	Pentachlorophenol content (mg/100 g tissue or mg/100 ml liquid)	
	Control	30-g pentachlorophenol solution
Blood	0.10	4.20
Fat	1.00[a]	<0.05[b]
Feces		
24-hr sample		0.60
48-hr sample		3.06
Kidney	0.10	0.60
Liver	0.60	0.58
Muscle	0.20	0.10
Urine		
24-hr sample		18.4
48-hr sample		21.2

[a] Bad sample consisting of hair, hide, and fat; poorly homogenized.

[b] Amounts less than 0.05 mg/100 g were not detectable.

Source: Adapted from Walters, 1952, Table 2, p. 305. Reprinted by permission of the publisher.

TABLE D.5.3. VARIABILITY OF PENTACHLOROPHENOL LEVELS IN BLOOD OF FIVE SHEEP FORCE-FED PENTACHLOROPHENOL-IMPREGNATED SAWDUST

Time after dose (hr)	Pentachlorophenol level in blood (mg/liter) at doses of				
	111 mg/kg	125 mg/kg	139 mg/kg	139 mg/kg	167 mg/kg
2	30	25	40	40	60
4	50	[a]	40	80	[b]
8	45		38	80	
12	40		[c]	60	
16	35			35	
24	30			5	

[a] Died in 4 hr.

[b] Died in 2.5 hr.

[c] Died in 12 hr.

Source: Compiled from Harrison, 1959.

mammals. Although transport and distribution of pentachlorophenol in domestic animals have not been extensively studied, some data are available. Harrison (1959) studied levels of pentachlorophenol in the blood of sheep force-fed pentachlorophenol-impregnated sawdust (Table D.5.3). The liver of one sheep which died after ingesting 125 mg pentachlorophenol per kilogram body weight contained 4 mg pentachlorophenol per 100 g wet tissue (40 ppm). Walters (1952) investigated the tissue distribution and excretion of pentachlorophenol in swine force-fed a solution of the compound. The distribution of pentachlorophenol in the organs, feces, and urine of these swine is shown in Table D.5.2. The kidney, which showed a higher retention of pentachlorophenol than any other tissue, contained six times more pentachlorophenol than the kidney of the control animal. Only small amounts of pentachlorophenol were found in other tissues as compared with levels in the control animal.

D.5.1.1.3 Biotransformation — Information on the metabolism of pentachlorophenol in birds and wild or domestic mammals is not available. Considerable data are available, however, on the metabolic products of pentachlorophenol degradation in experimental animals. This information (as well as a proposed integrated pathway for pentachlorophenol metabolism in mammals) is fully discussed in Section D.6.1.3. In summary, pentachlorophenol conjugates (including glucuronide conjugates), tetrachlorohydroquinone, tetrachlorohydroquinone conjugates, and chloranil appear to be major products of pentachlorophenol metabolism in mammals. Metabolism probably takes place primarily in the liver.

D.5.1.1.4 Excretion — Walters (1952) discussed the excretion of pentachlorophenol by domestic animals. Large amounts of pentachlorophenol recovered in the urine and feces of animals force-fed pentachlorophenol indicated that the substance is rapidly excreted (Table D.5.2). Most pentachlorophenol was excreted in the urine with smaller amounts present in the feces. The 48-hr feces sample contained much more pentachlorophenol than the 24-hr sample. Although more pentachlorophenol was excreted in the 48-hr than in the 24-hr urine sample, the amounts were similar. Urinary excretion, at least over 48 hr, appeared to be relatively constant. Fecal excretion appeared to peak at approximately 48 hr following ingestion.

Zumwalt et al. (1977) determined the uptake and elimination of pentachlorophenol in dairy cows following 14 daily oral exposures of 0.05, 0.5, or 5.0 mg/kg. Blood levels increased rapidly within 6 hr after dosing and reached a plateau in approximately 6 days. Plateau values were 0.054 mg/liter (0.05 mg/kg dose), 0.494 mg/liter (0.5 mg/kg dose), and 2.432 mg/liter (5.0 mg/kg dose). Pentachlorophenol levels in serum decreased rapidly when dosing stopped. The calculated half-life was less than 2 days. A linear relationship was found when log dose was plotted against log plasma level after 14 days of exposure.

D.5.1.2 Effects

D.5.1.2.1 Physiological or Biochemical Role — No data suggest that pentachlorophenol plays any normal physiological or biochemical role in birds or mammals.

D.5.1.2.2 Toxicity — Both pentachlorophenol and sodium pentachlorophenate are toxic when tested in many animals. The factors which primarily determine whether or not systemic toxicity will ensue are the dose and the ability of the animal to excrete the substance. If doses are high enough, pentachlorophenol is acutely toxic following a single administration; it exhibits chronic toxicity when administered by any of three principal routes.

D.5.1.2.2.1 Mechanism of action — The mechanism of toxic action of pentachlorophenol has been extensively studied in experimental animals. These data are presented in Section D.6.2.2.1. It is assumed that the data derived from experimental animal studies is directly applicable to domestic mammals and birds which are exposed to pentachlorophenol. In summary, pentachlorophenol uncouples oxidative phosphorylation, thus short-circuiting the energy metabolism of the animal. At high dosages, inhibition of other enzyme systems and gross cellular degeneration occur.

D.5.1.2.2.2 Local and systemic pathology — Application of sodium pentachlorophenate powder to the skin of rabbits caused marked irritation and even a chemical burn. Conversely, pentachlorophenol powder caused only slight skin redness in the rabbit (Dow Chemical Company, 1969*a*, 1969*b*). However, pentachlorophenol in petroleum solvents has caused serious skin damage and necrosis in experimental animals. Sows confined to farrowing crates with wooden platforms to which undiluted pentachlorophenol was applied developed extensive abdominal burns and necrosis following a 24-hr confinement (Schipper, 1961). A 2% pentachlorophenol solution in creosote applied to the clipped skin of calves caused marked changes which persisted for four weeks (Olafson and Leutritz, 1959).

Several cases of systemic toxicity of pentachlorophenol to farm animals have been reported. Symptoms of severe pentachlorophenol poisoning are hyperpyrexia, depression, rapid respiration, weakness, death without struggle, and rapid onset of rigor mortis following death (Spencer, 1957; Schipper, 1961; Blevins, 1965).

In experiments conducted by Schipper (1961), swine were confined to farrowing pens which had been freshly treated with a pentachlorophenol formulation. Neonatal mortality among the litters of swine confined to the treated pens was extensive. Autopsy results of the fatally poisoned animals indicated the following pathological changes: severe lesions of the kidney including subcapsular fluid and hemorrhages, congestion of the liver and spleen, congestion and inflammation of the stomach and intestinal tract along with the consistent presence of large amounts of gas, necrosis of the respiratory tract as well as congestion and mild emphysema, an abnormal fluid in the urinary bladder which was white, tenacious, and contained fibrinous threads, and, finally, various local lesions on the skin and in the oral cavity.

Harrison (1959) administered lethal doses of pentachlorophenol to sheep and calves in the form of pentachlorophenol-impregnated sawdust. Postmortem findings included enlarged edematous lymph nodes; collapsed and generalized congestion of the lungs; mild congestion (in some animals) of the stomach, intestines, liver, and kidney; and, occasionally, hemorrhages in the epicardium and along the aorta. Pathological changes in pentachlorophenol-poisoned experimental animals and humans are discussed on the basis of individual organs in Section D.6.2.2.2.

D.5.1.2.2.3 Acute toxicity — A limited number of reports on the acute toxicity of pentachlorophenol to domestic mammals and wildlife are available. Minimum lethal doses and LD_{50} values have been reported for experimental animals (Section D.6.2.2.3).

Spencer (1957) discussed the fatal poisonings of two Hereford cows. Drinking water in the pasture where the fatalities occurred was at a considerable distance, but an open barrel of 5% pentachlorophenol in kerosene was found at the scene. The diagnosis was poisoning caused by the consumption of pentachlorophenol in kerosene. Because both kerosene and pentachlorophenol are toxic, the exact contribution of pentachlorophenol to the deaths is unclear. Another case of livestock poisoning involved swine confined to farrowing pens treated with pentachlorophenol. Ten neonatal pigs confined for 24 hr in a farrowing pen treated with pentachlorophenol as a wood preservative died (Blevins, 1965). The owners had improperly treated the farrowing pens with excessive doses of pentachlorophenol, and no bedding was provided, allowing constant cutaneous contact with the pentachlorophenol-treated wood. Autopsy results revealed grossly congested and distended lungs and pronounced cardiac and renal petechiation.

The investigations undertaken by Schipper (1961) were prompted by extensive losses of baby pigs in the immediate area of his laboratory. The hazard involved in confining swine to pens freshly treated with pentachlorophenol was demonstrated conclusively. In these experiments, swine were confined to farrowing pens which had been treated with a 4% solution of pentachlorophenol in petroleum distillate. Pig mortalities were high and increased with length of confinement. Neonatal mortality among the litters confined to pentachlorophenol-treated farrowing pens was extensive. The surviving animals showed a pronounced inability to gain weight until they were approximately five to six weeks old (Table D.5.4). This low weight gain was attributed to continuous cutaneous uptake of pentachlorophenol from the farrowing pen, inadequate nursing due to pentachlorophenol contamination of the mothers' teats, and direct pentachlorophenol ingestion.

Harrison (1959) studied the toxicity of pentachlorophenol to calves and sheep (for chronic toxicity data, see Section D.5.1.2.2.4). Because the purpose of this investigation was to evaluate the hazard posed by pentachlorophenol-treated wood, pentachlorophenol was administered to animals in the form of impregnated sawdust. Dry Douglas fir (*Pseudotsuga taxifolia*) sapwood was "loaded" with pentachlorophenol at a rate of 320 kg of pentachlorophenol solution per cubic meter of wood. The solution contained 5% pentachlorophenol in a petroleum solvent. Standard loading for larch (*Larix* sp.) and Douglas fir posts is 112 kg/m^3; therefore, a loading of 320 kg/m^3 was adopted to approximate the amount in the outside layers of posts (the region which is most likely to be ingested by animals).

TABLE D.5.4. COMPARISON OF AVERAGE WEIGHT OF PIGS INVOLVED IN EXPERIMENTS TO DETERMINE TOXICITY OF PENTACHLOROPHENOL

Treatment	Number of pigs	Average weight (kg)			
		Birth	7 days old	21 days old	42 days old
Farrowing pens treated with 4% pentachlorophenol-petroleum distillate formulation	141	1.54	2.77	5.58	10.99
Control	36	1.44	3.76	9.39	11.49

Source: Adapted from Schipper, 1961, Table 2, p. 404. Reprinted by permission of the publisher.

The minimum acute lethal dose of pentachlorophenol to sheep was approximately 120 mg pentachlorophenol per kilogram body weight. The acute lethal dose for calves was estimated to be 140 mg pentachlorophenol per kilogram body weight. Acute poisoning resulted in deaths from 2 to 14 hr following onset of symptoms. The most prominent symptom reported was an accelerated breathing rate. Badly affected animals swayed, panted noisily with head lowered, and made little or no attempt to move away when approached. Recovery was rapid and complete if force-feeding was stopped at this stage of poisoning. In fatal cases, complete collapse occurred; the animals lay limp and panted vigorously through open mouths. Rigor mortis set in with extreme rapidity, and the body became completely rigid within 1 or 2 min after death. Proteinuria and the presence of reducing sugars in the urine were reported for some animals. Postmortem findings included enlarged edematous lymph nodes, collapse and generalized congestion of the lungs, mild congestion (in some animals) of the stomach, intestines, liver, and kidney, and occasionally, hemorrhages in the epicardium and along the aorta.

Extensive loss of wildlife was reported by Vermeer et al. (1974) following the widespread application of sodium pentachlorophenate as a molluscicide in Surinam, South America. The incident occurred on an 8000-ha rice-growing project at Wageningen in the western coastal region of Surinam. The sodium pentachlorophenate was applied widely to the rice fields for control of water snails (*Pomacea glauca* and *P. lineata*) which damage young rice plants. A total of 50,000 kg was applied to the rice fields from 1965 to 1971, giving a pentachlorophenol concentration of approximately 4 kg/ha. An investigation took place from October to December 1971 following the extensive spraying of sodium pentachlorophenate. The deaths of fish, frogs, snails, and birds were recorded, and the pentachlorophenol residues in the dead organisms were determined (Table D.5.5). Concentrations of pentachlorophenol in dead fish ranged from 31 to 59 mg/kg. Residue levels of 37 mg/kg were found in dead snails. Concentrations of pentachlorophenol in fish collected live from unsprayed ditches were also substantial, ranging from 2 to 13 mg/kg.

TABLE D.5.5. PENTACHLOROPHENOL IN COMPOSITE SAMPLES OF SNAILS, FROGS, AND FISH COLLECTED IN RICE FIELDS AT WAGENINGEN, SURINAM, NOVEMBER 1971

Species	Sample description	Number of animals	Fat in animals (%)	Pentachlorophenol residue (µg/g wet wt)
Snails (*Pomacea*)	Dead after sodium pentachlorophenate application	10	4.0	36.8
Frog (*Pseudis paradoxa*)	Dead after sodium pentachlorophenate application	6	5.2	8.1
Fish				
Kwi kwi (*Hoplosternum littorale*)	Dead after sodium pentachlorophenate application	8	7.9	41.6
	Alive from unsprayed ditches	5	6.4	13.4
Srieba (*Astyanax bimaculatus*)	Dead after sodium pentachlorophenate application	8	7.5	59.4
	Alive from unsprayed ditches	10	8.4	1.77
Krobia (*Cichlasoma bimaculatum*)	Dead after sodium pentachlorophenate application	8	5.2	31.2
	Alive from unsprayed ditches	8	5.2	8.76

Source: Adapted from Vermeer et al., 1974, Table 8, p. 228. Reprinted by permission of the publisher.

Vermeer et al. (1974) also reported that 50 snail kites (birds that feed almost exclusively on snails) were found dead between November 28 and December 4, 1971, after a period of intensive application of sodium pentachlorophenate in November. It was concluded that the kites died from pentachlorophenol poisoning. Seventeen of the 50 dead birds were sampled for pentachlorophenol residues in brain, liver, and kidney (Table D.5.6). The organochlorine insecticides — DDT, dieldrin, and endrin — were present at very low or undetectable concentrations, but the mean concentration of pentachlorophenol was comparatively high. Living birds were also analyzed for pentachlorophenol content. Table D.5.7 gives the residue levels in birds found foraging in the rice fields as well as values for birds which were seen foraging in an untreated freshwater marsh. The difference in pentachlorophenol levels in tissues is evident. Other species of birds that habitually frequented the rice fields were also sampled (Table D.5.8). All of the samples contained detectable concentrations of pentachlorophenol. The death of the snail kites resulted from their consumption of snails containing pentachlorophenol. The substantial levels of pentachlorophenol noted in other birds indicated that pentachlorophenol contamination of wildlife in the area was fairly widespread. The effect of these sublethal pentachlorophenol levels in wildlife was not determined. The use of sodium pentachlorophenate to control snails makes neither economic nor ecological sense because pentachlorophenol apparently poses a hazard to snail kites, a species of bird which feeds exclusively on snails that damage young rice plants. Thus, the use of sodium pentachlorophenate interferes with rather than reinforces the natural predation on snails. Vermeer et al. (1974) recommended that, despite the high cost, Bayluscide (a 1:1 mixture of 2,5-dichloro-4-nitrosalicylanilide and 2-aminoethanol) be substituted whenever possible for sodium pentachlorophenate.

Hill et al. (1975) determined the dietary toxicity of pentachlorophenol in four species of young birds. Pentachlorophenol was added to the diets at various levels, and the LC_{50} values were determined after

TABLE D.5.6. PESTICIDE RESIDUES IN 17 SNAIL KITES FOUND DEAD AT A ROOST ADJACENT TO RICE FIELDS AT WAGENINGEN, SURINAM, NOVEMBER 27-28, 1971

Number of birds	Tissue analyzed	Fat in tissue (%)	Mean residue ± standard error (µg/g tissue wet wt)			
			Total DDT	Dieldrin	Endrin	Pentachlorophenol
17	Brain	4.83 ± 0.88	<0.01	<0.01	<0.01	11.25 ± 1.11
17	Liver	4.29 ± 0.58	0.01 ± 0.006	0.03 ± 0.01	<0.01	45.56 ± 2.18
10	Kidney	1.35 ± 0.31	<0.01	<0.01	<0.01	20.34 ± 1.25

Source: Adapted from Vermeer et al., 1974, Table 10, p. 230. Reprinted by permission of the publisher.

TABLE D.5.7. COMPARISON OF PENTACHLOROPHENOL RESIDUES IN SNAIL KITES FORAGING IN PENTACHLOROPHENOL-TREATED RICE FIELDS AND THOSE IN A FRESHWATER MARSH AT WAGENINGEN, SURINAM, NOVEMBER 29 – DECEMBER 3, 1971

Number of birds	Tissue analyzed	Foraged in rice fields		Foraged in freshwater marsh	
		Fat in tissue (%)	Pentachlorophenol content (μg/g wet wt)	Fat in tissue (%)	Pentachlorophenol content (μg/g wet wt)
3	Brain	4.2	2.11	6.0	0.04
3	Liver	2.0	10.4	2.7	0.14
3	Kidney	1.1	16.6	0.6	0.10

Source: Adapted from Vermeer et al., 1974, Table 11, p. 231. Reprinted by permission of the publisher.

five days of feeding. The LC_{50} values reported were as follows: bobwhite, about 3400 ppm; Japanese quail, 5204 ppm; ring-necked pheasant, 4331 ppm; and mallard duck, about 4500 ppm. These values fell in categories IV and V in a five-level classification system; class V is regarded as practically nontoxic. By comparison, dieldrin was 24 to 87 times more toxic in this test system.

D.5.1.2.2.4 Chronic toxicity – Investigations have been made of the systemic effects and levels of pentachlorophenol in the blood and urine following chronic or repeated doses of the compound or its sodium salt to domestic animals. Harrison (1959) studied the chronic toxicity of pentachlorophenol-impregnated sawdust on calves and sheep (for acute toxicity data, see Section D.5.1.2.2.3). Dry Douglas fir (*Pseudotsuga taxifolia*) sapwood was impregnated with 320 kg of a 5% pentachlorophenol solution per cubic meter of wood. Animals were daily force-fed impregnated sawdust and were observed for signs of systemic toxicity. Adverse cumulative effects in sheep as reflected by loss of body weight and deterioration in general condition were caused by daily intakes of 27.8 to 55.6 mg pentachlorophenol per kilogram body weight (Table D.5.9). The lowest daily dose rate which caused noticeable systemic effects was 27.8 mg/kg. In a similar test, the minimum toxic dose rate of pentachlorophenol to calves was estimated to be 35 mg/kg body weight (Table D.5.10). Studies of body weight changes in sheep were inconclusive. One animal receiving a daily dose of 13.9 mg/kg gained approximately 5 kg over an 18-day period. The control animal gained approximately 18 kg over this same period. A third animal receiving 27.8 mg/kg daily gained approximately 22 kg.

Olafson and Leutritz (1959) tested the effect of a creosote-pentachlorophenol mixture on cattle. A formulation of 2% technical pentachlorophenol in creosote was fed to cattle for two months. A 135-kg

TABLE D.5.8. PENTACHLOROPHENOL RESIDUES IN COMPOSITE TISSUE SAMPLES OF BIRDS AND SPECTACLED CAIMAN COLLECTED ON RICE FIELDS AT WAGENINGEN, SURINAM, OCTOBER 21-31, 1971

Species	Number of birds	Tissue analyzed	Fat in tissue (%)	Penta-chlorophenol content (μg/g wet wt)
Snail kite (*Rostrhamus sociabilis*)	5	Brain	6.5	0.10
	5	Liver	2.5	0.19
Black vulture (*Coragyps atratus*)	5	Brain	4.8	0.09
	5	Liver	3.1	0.06
Common egret (*Egretta alba*)	10	Brain	6.0	0.08
	10	Liver	2.8	0.14
Snowy egret (*Egretta thula*)	10	Brain	5.2	0.10
	10	Liver	2.0	0.19
Cattle egret (*Ardeola ibis*)	10	Brain	5.5	0.49
	10	Liver	3.2	0.07
Purple gallinule (*Porphyrula martinica*)	10	Brain	6.0	0.10
	10	Breast muscle	4.8	0.04
Spectacled caiman (*Caiman crocodilus*)	10	Brain	7.2	0.18
	10	Liver	4.4	0.24

Source: Adapted from Vermeer et al., 1974, Table 7, p. 226. Reprinted by permission of the publisher.

calf was able to tolerate 0.45 kg of this formulation over a two-month period without showing appreciable detrimental effects. Grigsby and Farwell (1950) tested the effect on livestock of pentachlorophenol in a mineral oil emulsion used as a herbicide on grazing land. Horses, dairy and beef cattle, sheep, and swine avoided areas sprayed with a pentachlorophenol formulation; the animals invariably preferred grazing on unsprayed pasture. These authors therefore concluded that the use of pentachlorophenol as a herbicide on pastureland is a relatively safe procedure because livestock preferentially avoid sprayed areas.

Herdt, Loomis, and Nolan (1951) tested the effect of sodium pentachlorophenate on cattle. The investigation was undertaken to test the feasibility of using sodium pentachlorophenate as a molluscicide. The substance was administered to three young bulls in drinking water at daily dose rates of 7.6 mg/kg for a minimum of five weeks. Pulse, respiration rate, temperature, urine analyses, and blood counts were monitored, and no significant deviations from normal were found. Furthermore, no toxic manifestations were evident on autopsy of the animals. They concluded that the use of sodium pentachlorophenate as a molluscicide in the field is a relatively safe procedure if reasonable precautions are taken in its application.

The effect on swine, sheep, and cattle of preservative-treated gates, watering troughs, feed troughs, salt boxes, posts, electrical fence stakes, and corral timbers was tested by Walters (1952). The

TABLE D.5.9. CHRONIC TOXICITY DOSE RATES AND EFFECTS OF PENTACHLOROPHENOL ON TREATED SHEEP

Daily dose of pentachlorophenol (mg/g)	Daily dose of pentachlorophenol (g)	Weight of treated sawdust (g)	Number of doses	Effect
13.9	0.32	13	19	None
27.8	0.71	29	19	Slight
55.6	1.50	61	19	Symptoms and severe loss of body weight
0	0	84	12	None

Source: Adapted from Harrison, 1959, Table 2, p. 90. Reprinted by permission of the publisher.

TABLE D.5.10. CHRONIC TOXICITY DOSE RATES AND EFFECTS OF PENTACHLOROPHENOL ON TREATED CALVES

Daily dose of pentachlorophenol (mg/kg)	Daily dose of pentachlorophenol (g)	Weight of treated sawdust (g)	Number of doses[a]	Effect
35	4.16	168	11	Temporary check in growth
50	6.60	267	9	Severe loss of weight and in general condition
70	10.90	442	2	Died 4 hr after second dose

[a] Limited by the sawdust available.

Source: Adapted from Harrison, 1959, Table 4, p. 91. Reprinted by permission of the publisher.

treated items were cold-soaked in the preservative solution consisting of 5% pentachlorophenol in a mineral spirit solvent for a maximum of 48 hr. The sheep exposed to the treated equipment for two weeks showed no ill effects. No attempt was made to determine whether or not pentachlorophenol entered the animal by ingestion. Exposure of calves to the treated wood for one month apparently caused no detrimental effects. Again, pentachlorophenol levels in the bodies of the animals were not determined. The length of time these animals were exposed to the pentachlorophenol-treated equipment does not appear to be sufficient to evaluate long-term effects.

D.5.1.2.3 Carcinogenicity, Teratogenicity, and Mutagenicity — No information is available on possible carcinogenic, teratogenic, or mutagenic effects of pentachlorophenol in birds and domestic mammals. A tumor-promoting effect of pentachlorophenol following application of dimethyibenzanthracene to the skin of mice has been reported (Section D.6.2.3), but it is not known whether this effect will occur in birds or domestic animals.

D.5.1.2.4 Musty Taint — A problem of economic importance has recently surfaced in the broiler chicken industry in England. The problem involves the presence of a musty taint in chicken and chicken eggs. Curtis et al. (1972, 1974) have found that the cause of this musty taint is the presence of 2,3,4,6-tetrachloroanisole and pentachloroanisole in broiler chickens. The anisoles are found in substantial quantities in wood shavings used as bedding in the cages of chickens. They apparently are formed in the litter as a result of methylation of the parent chlorophenols (including pentachlorophenol) by fungi present in the litter. Although the primary offender causing musty taint appears to be tetrachloroanisole, pentachloroanisole may be the causative agent at much higher concentrations (1000 times). The widespread use of pentachlorophenol and tetrachlorophenol as wood preservatives apparently results in the presence of a substantial amount of these compounds in wood shavings used for bedding of chickens. It has also been suggested that tetrachlorophenol may arise from dechlorination of pentachlorophenol (Parr et al., 1974). Odor thresholds for pentachloroanisole and tetrachloroanisole are remarkably low (Table D.5.11). Samples of litter where musty taint of chickens has been noted showed levels of pentachloroanisole and tetrachloroanisole to be 5 mg/kg. The mode of entry of these compounds into the birds has not been established (Curtis et al., 1972). Pentachlorophenol and tetrachlorophenol were found in freshly sawn lumber at levels of 40 to 100 mg/kg. Parr et al. (1974) did not favor the view that prevalent concentrations of tetrachlorophenol in wood shavings result from dechlorination of pentachlorophenol (Table D.5.12). Rather, they believed that the prevalence of tetrachlorophenol in wood shavings is due to the more common use of this compound as a wood preservative. They stated, "Although pentachlorophenol is generally thought to be more widely used than 2,3,4,6-tetrachlorophenol as a wood preservative this is not supported by these results. Technical grade pentachlorophenol contains 10 to 20% of 2,3,4,6-tetrachlorophenol (and the converse is true) but neither this nor the variable recoveries obtained for pentachlorophenol would explain the values observed. It is possible, but unlikely, that some selective conversion of pentachlorophenol to 2,3,4,6-tetrachlorophenol occurs at some stage before the shavings are sampled but it seems more likely that the use of 2,3,4,6-tetrachlorophenol as a preservative against sap-stain is greater than is generally realised." Removal of chickens from contaminated litter several days before slaughter prevents musty taint (Curtis et al., 1974). Curtis et al. (1972) commented, "In this case the extremely low sensory thresholds of the

TABLE D.5.11. DETECTION THRESHOLDS FOR CHLOROANISOLES IN AQUEOUS SOLUTION[a]

Compound	Threshold concentration (μg/g solution)
Pentachloroanisole	4×10^{-3}
2,3,4,6-Tetrachloroanisole	4×10^{-6}
2,4,6-Trichloroanisole	3×10^{-8}
2,3,6-Trichloroanisole	3×10^{-10}

[a] Tests were made with 23 subjects in triplicate and are significant at the 1% level.

Source: Adapted from Curtis et al., 1972, Table 1, p. 223. Reprinted by permission of the publisher.

TABLE D.5.12. CHLOROPHENOL AND CHLOROANISOLE LEVELS IN FRESH SHAVINGS AND SPENT LITTER FROM BROILER CHICKEN HOUSES

Sample	Compound	Chlorophenol or chloroanisole level (μg/g wet wt)	
		Mean	Range
Fresh shavings	Pentachlorophenol	12	0.6-83
	2,3,4,6-Tetrachlorophenol	54	4-310
Spent litter	Pentachlorophenol	0.3	0-4.1
	2,3,4,6-Tetrachlorophenol	0.7	0-5.6
	2,3,4,6-Tetrachloroanisole	0.5	0-3.6
	Pentachloroanisole	0.03	0-0.3

Source: Adapted from Parr et al., 1974, Table 1, p. 838. Reprinted by permission of the publisher.

chloroanisoles...are fortunate because they probably discourage the consumption of chickens containing these compounds, the toxicities of which have not yet been established."

D.5.2 BIOLOGICAL ASPECTS IN FISH AND OTHER AQUATIC ORGANISMS

D.5.2.1 Metabolism

D.5.2.1.1 Uptake and Absorption — Fish and other aquatic organisms can absorb pentachlorophenol through the gills, the gastrointestinal tract, or directly through the body surfaces; absorption through the gills and ingestion are the most likely routes of uptake. Because it is impossible to differentiate between pentachlorophenol that enters the fish as a result of absorption from the water and that which is ingested, these two routes of uptake are considered together. No studies which discussed uptake of pentachlorophenol via ingestion by fish or aquatic organisms were found; therefore, it is assumed that the effects noted following uptake of the compound from the water apply to all routes of uptake.

Pentachlorophenol accumulates in aquatic organisms (Rudling, 1970). Tissue levels 100 to 1000 times the levels of ambient pentachlorophenol in water have been found in fish taken from a lake into which pulp mill effluents were discharged (Table D.5.13). The eel, a species with a high fat content, acquires higher levels of pentachlorophenol than species with a more normal fat content. The propensity of pentachlorophenol, and other organochlorine compounds, to accumulate in the fatty tissue of aquatic organisms is of serious concern because this allows the buildup of toxic substances throughout the food chain. Rainbow trout readily remove pentachlorophenol from the surrounding water (Tsuda and Kariya, 1963) (Figure D.5.1). The short-necked clam (*Tapes philippinarum*) (Kobayashi, Akitake,

TABLE D.5.13. PENTACHLOROPHENOL LEVELS IN FISH AND WATER DOWNSTREAM FROM A PULP MILL

Sample	Pentachlorophenol content	
	(μg/liter)	(μg/kg)
Water		
Immediately downstream from mill	9	
Receiving lake	3	
Fish		
Pike (*Esox lucius*)		200
Perch (*Perca fluviatilis*)		150
Eel (*Anguilla anguilla*)		3000

Source: Adapted from Rudling, 1970, Table 5, p. 536. Reprinted by permission of the publisher.

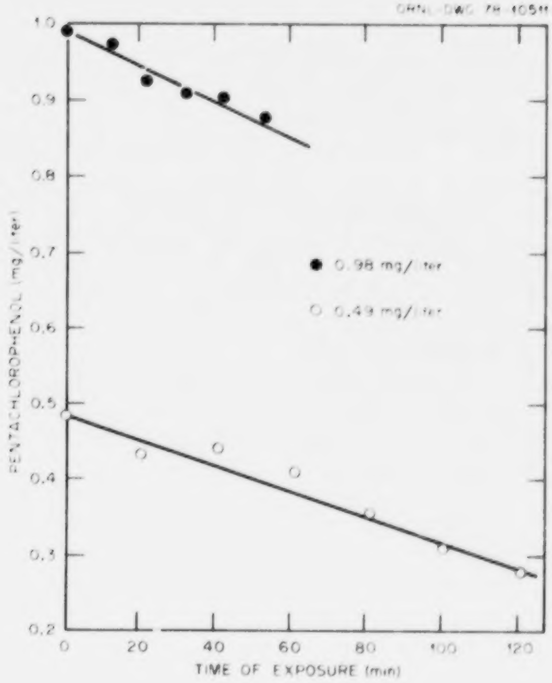

Figure D.5.1. Decrease of pentachlorophenol concentration in water during culture of rainbow trout. Source: Adapted from Tsuda and Kariya, 1963, Figure 1, p. 830. Reprinted by permission of the publisher.

and Tomiyama, 1969), the eel (*Anguilla anguilla* L.) (Holmberg et al., 1972), and the goldfish (*Carassius auratus*) (Kobayashi and Akitake, 1975*a*) also remove pentachlorophenol.

A rapid decrease in pentachlorophenol concentration during culture of the short-necked clam was not accompanied by a quantitatively equal accumulation in this shellfish. Furthermore, pentachlorophenol levels in the shellfish decreased with time without a concomittant increase in the concentration of pentachlorophenol in the water (Kobayashi, Akitake, and Tomiyama, 1970*a*). These results can be explained by a detoxification mechanism in the shellfish (Section D.5.2.1.3).

Kobayashi and Akitake (1975*a*) exposed goldfish to [^{14}C]sodium pentachlorophenate at levels of 0.1, 0.2, and 0.4 mg/liter (Figure D.5.2). Fish exposed to 0.1 mg/liter showed no evidence of adverse effects, although pentachlorophenol concentrations increased with time, reaching a peak of 90 μg/g. This increase represents a concentration factor from the original pentachlorophenol medium of 1000 following a 120-hr exposure. The fish exposed to 0.2 mg/liter pentachlorophenol showed a similar accumulation with time; however, accumulation was more rapid. After the concentration in the fish reached a level of about 100 μg pentachlorophenol per gram body weight, some of the fish died. No surviving fish

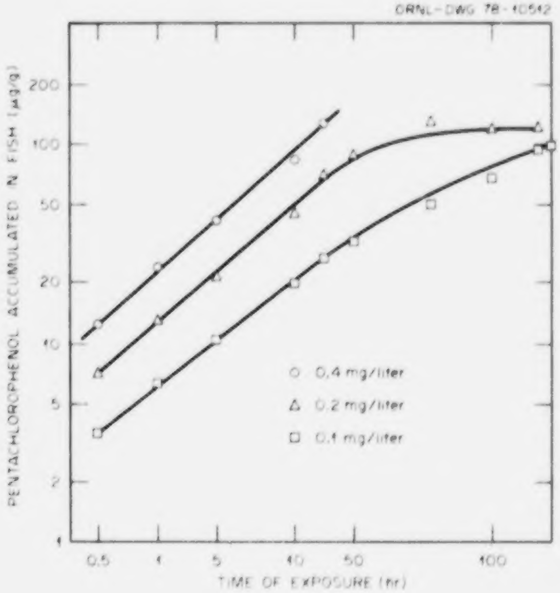

Figure D.5.2. Accumulation of pentachlorophenol in goldfish surviving in pentachlorophenol media (0.1, 0.2, and 0.4 mg/liter). Source: Adapted from Kobayashi and Akitake, 1975*a*, Figure 1, p. 89. Reprinted by permission of the publisher.

showed pentachlorophenol concentrations higher than 114 µg/g. Those exposed to 0.4 mg/liter showed an increase in pentachlorophenol concentration with time, reaching a peak of 111 µg/g after 6 hr; deaths occurred within 4 hr of exposure at this level, and no fish survived more than 6 hr.

D.5.2.1.2 Transport and Distribution — The distribution of absorbed pentachlorophenol in the tissues of the short-necked clam (*Tapes philippinarum*) was studied by Kobayashi, Akitake, and Tomiyama (1969). Although pentachlorophenol was absorbed rapidly by the shellfish and distributed to various tissues, especially the bojanus' organ, liver, and digestive tract, it was rapidly eliminated, presumably from the bojanus' organ. Elimination by the bojanus' organ was inferred from the tissue distribution pattern (Table D.5.14). The bojanus' organ rapidly accumulated pentachlorophenol, and high levels of the compound were maintained throughout a 24-hr excretion period. On the other hand, the liver and digestive tract contained high levels of pentachlorophenol during the exposure period, but the level rapidly decreased during the excretion phase in normal seawater. Rapid elimination occurred, and pentachlorophenol concentration in the whole body decreased sixfold over the 24-hr excretion phase.

The distribution of sodium pentachlorophenate in eel tissues was studied by Holmberg et al. (1972). Eels (*Anguilla anguilla*) in either

TABLE D.5.14. DISTRIBUTION OF PENTACHLOROPHENOL IN THE SHORT-NECKED CLAM (*TAPES PHILIPPINARUM*)

Organ	Pentachlorophenol content							
	Seawater with [^{14}C]pentachlorophenol				Normal seawater[a]			
	24 hr		48 hr		8 hr		24 hr	
	(μg/g)	(% of whole-body burden)	(μg/g)	(% of whole-body burden)	(μg/g)	(% of whole-body burden)	(μg/g)	(% of whole-body burden)
Bojanus' organ	70	7	85	10	53	23	32	23
Liver	20	8	10	10	4	10	4	20
Digestive tract	19	7	10	8	2	5	0.5	1
Gills	8	11	4	12	1.8	14	1	9
Remainder	8	67	3	60	1.0	48	0.8	47
Whole body	9	100	4	100	2.0	100	1.5	100

[a] Cultured in seawater treated with [^{14}C]pentachlorophenol; transferred at 48 hr to normal seawater.

Source: Compiled from Kobayashi, Akitake, and Tomiyama, 1969.

freshwater or saltwater were exposed to 0.1 mg/liter sodium pentachlorophenate and were subsequently transferred to a medium free of pentachlorophenol. Levels of pentachlorophenol in the tissues were monitored during the recovery period (Figure D.5.3). The highest pentachlorophenol levels were found in the liver of eels exposed either to freshwater or saltwater. A positive relationship was found between exposure time and maximum levels of pentachlorophenol in the tissues (4 days in freshwater and 8 days in seawater). During the recovery period for the freshwater eels, levels of pentachlorophenol in the tissues decreased; however, treated animals still had about 1.2 μg/g in the liver and 0.08 μg/g in the muscle after 55 days. These results do not prove conclusively, however, that pentachlorophenol has a long half-life in eels. This experiment lacked two crucial controls. The untreated water was not analyzed for pentachlorophenol content. From previous reports of the ability of fish and eels to concentrate pentachlorophenol by 200 to 1000 times, trace amounts of contamination could possibly account for part of the tissue residues at the end of the trial. Also, although control eels were placed in the untreated water, tissue residues were not reported. Additionally, freshwater and saltwater eels reportedly contained pentachlorophenol in tissues before the experiment started. Levels were 1.0 mg/kg in the liver and 0.2 mg/liter in the blood of seawater eels and 0.08 mg/kg in the liver and 0.02 mg/liter in the blood of freshwater eels. Although differences in rates of uptake in the two

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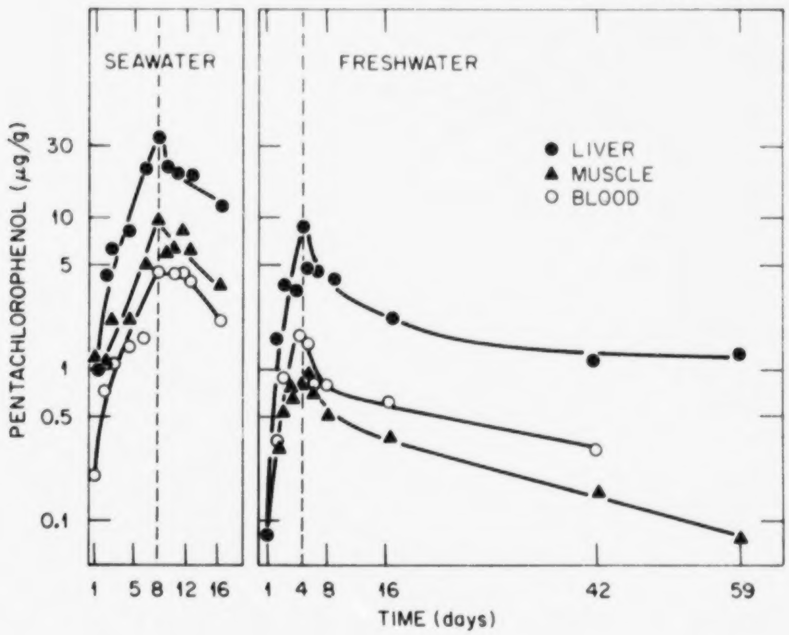

Figure D.5.3. Pentachlorophenol levels in various tissues of eels from seawater and freshwater tests. Source: Adapted from Holmberg et al., 1972, Figure 1, p. 175. Reprinted by permission of the publisher.

media were not clear, the authors suggested that the more rapid action in freshwater may have resulted from the difference in pH between seawater and freshwater. The pH of water is known to affect ionization of phenols, which would thereby affect pentachlorophenol uptake. The lipids, where most of the stored pentachlorophenol appeared, were suggested as a source for pentachlorophenol redistribution.

Kobayashi and Akitake (1975*b*) studied the turnover of absorbed pentachlorophenol in various tissues of the goldfish (*Carassius auratus*). Pentachlorophenol levels in the tissues were examined during active absorption from a pentachlorophenol-containing medium, and subsequent excretion was determined with the fish in running water. Goldfish were placed in a medium containing [^{14}C]pentachlorophenol (pentachlorophenol at 0.2 mg/liter); the fish were then removed at 1, 5, 12, and 24 hr following initiation of treatment, and selected tissues were analyzed for pentachlorophenol (Table D.5.15). Other fish subjected to a 24-hr exposure to pentachlorophenol were placed in running water, and tissue samples were analyzed after recovery periods of 1, 5, 12, and 24 hr (Table D.5.16). Pentachlorophenol accumulation in the gallbladder reached values of 539 μg/g following the 24-hr exposure. A further increase was seen even after the fish were transferred to clean running water for 24 hr. In contrast, the pentachlorophenol content of other tissues decreased following transfer to running water. Pentachlorophenol in the gallbladder eventually reached a peak of 1077 μg/g, which corresponded to a concentration factor of 5400 over the pentachlorophenol content of the medium. In fact, pentachlorophenol in the gallbladder accounted for 41% of the total pentachlorophenol detected in the fish after the transfer to running water for 24 hr. Tables D.5.15 and D.5.16 show that relatively high amounts of pentachlorophenol accumulated in the blood and the gills during early periods of exposure (19% and 8% respectively). These amounts subsequently declined to levels of 11% and 4% at the end of the excretion period. Amounts in the kidney and hepatopancreas, however, were almost constant during the absorption and excretion phases. A small increase in the specific activity of ^{14}C occurred in the gallbladder with time, indicating decomposition of some of the pentachlorophenol in the fish. The authors suggested that pentachlorophenol was detoxified following transfer to the hepatopancreas and was partly transported to the gallbladder and the bile. In a later study, Akitake and Kobayashi (1975*b*) demonstrated that pentachlorophenol is excreted by goldfish as a sulfate conjugate (Section D.5.2.1.4).

Glickman et al. (1977) demonstrated that rainbow trout exposed to 25 μg/liter pentachlorophenol for 24 hr accumulated the following levels in tissues: liver, 16 mg/kg; blood, 6.5 mg/liter; fat, 6.0 mg/kg; and muscle, 1 mg/kg. Elimination half-lives were as follows: blood, 6.2 hr; liver, 9.8 hr; fat, 23 hr; and muscle, 6.9 hr. Trout did not methylate pentachlorophenol (i.e., did not form pentachloroanisole which has a higher affinity for fat and a much longer tissue half-life). The only metabolite found in bile was the glucuronide conjugate.

Pruitt, Grantham, and Pierce (1977) found that bluegill (*Lepomis macrochirus*) placed in water containing 0.1 mg/liter pentachlorophenol

TABLE D.5.15. CHANGES IN AMOUNT OF PENTACHLOROPHENOL ACCUMULATED IN GOLDFISH TISSUES DURING EXPOSURE TO A MEDIUM CONTAINING 0.2 mg/liter [^{14}C]PENTACHLOROPHENOL

Tissue	Pentachlorophenol content at exposure time of							
	1 hr		5 hr		12 hr		24 hr	
	(μg/g tissue)	(% of whole-body burden)	(μg/g tissue)	(% of whole-body burden)	(μg/g tissue)	(% of whole-body burden)	(μg/g tissue)	(% of whole-body burden)
Gills	4.2	8.0	10	5.8	19	5.4	21	5.0
Blood	9.7	19.0	32	20.4	55	16.0	60	13.4
Hepatopancreas	2.4	7.8	11	10.2	18	9.1	26	9.2
Gallbladder	ND[a]	ND	43	3.3	216	5.7	534	13.8
Kidney	ND	ND	19	2.6	39	2.8	35	1.9
Spleen	ND	ND	ND	ND	17	0.4	28	0.5
Heart	ND	ND	17	0.8	29	0.5	29	0.4
Testis	ND	ND	2.7	0.6	7.4	2.3	11	0.7
Ovary	0.57	2.3	2.7	4.7	10	0.9	38	4.9
Air bladder	ND	ND	7.6	0.9	17	0.9	19	0.8
Digestive tract	1.5	3.6	8.9	5.8	23	6.4	29	7.4
Brain	ND	ND	9.8	0.7	14	0.6	18	0.6
Muscle	0.78	12.0	2.1	10.1	4.9	11.3	4.9	8.5
Vertebra	ND	ND	4.8	1.5	8.9	1.0	10	0.8
Skin	2.9	3.8	9.0	3.6	21	3.7	22	3.2
Scale	3.5	10.3	8.2	7.9	12	5.4	15	5.4
Remainder	1.2	33.3	2.3	20.9	6.1	27.8	6.3	23.3
Whole body	1.5		4.7		10		13	

[a] Not detectable.

Source: Adapted from Kobayashi and Akitake, 1975b, Table 1, p. 94. Reprinted by permission of the publisher.

TABLE D.5.16. CHANGES IN RETENTION OF PENTACHLOROPHENOL IN GOLDFISH TISSUES DURING CULTURE IN RUNNING WATER AFTER A 24-hr EXPOSURE TO A 0.2 mg/liter [^{14}C]PENTACHLOROPHENOL MEDIUM

Tissue	Pentachlorophenol content during excretion time of							
	1 hr		5 hr		12 hr		24 hr	
	(μg/g tissue)	(% of whole-body burden)	(μg/g tissue)	(% of whole-body burden)	(μg/g tissue)	(% of whole-body burden)	(μg/g tissue)	(% of whole-body burden)
Gills	19	4.6	18	4.0	15	4.5	13	3.8
Blood	42	10.0	42	10.5	37	12.0	34	11.4
Hepatopancreas	23	9.4	20	7.7	16	7.8	13	7.5
Gallbladder	461	9.5	648	14.7	665	21.1	1074	33.8
Kidney	44	2.2	40	2.6	32	2.5	28	2.1
Spleen	18	0.4	15	0.4	21	0.7	ND[a]	
Heart	28	0.4	25	0.4	23	0.5	15	0.3
Testis	10	1.4	6.5	2.0	5.3	1.8	4.4	1.3
Ovary	9.0	2.1					3.6	0.5
Air bladder	16	0.9	16	0.8	13	0.9	8.0	0.6
Digestive tract	52	13.8	34	10.0	25	8.0	21	6.9
Brain	18	0.6	15	0.6	10	0.6	8.1	0.4
Muscle	4.8	8.6	5.0	9.3	3.3	8.5	2.4	6.4
Vertebra	11	0.8	8.3	0.7	7.4	0.8	6.7	0.7
Skin	23	3.4	20	3.5	14	3.0	15	3.0
Scale	15	5.5	12	5.2	9.5	5.2	9.7	5.1
Remainder	6.9	26.5	6.7	27.7	4.3	22.2	3.0	16.2
Whole body	13		12		9.4		8.9	

[a] Not detectable.

Source: Adapted from Kobayashi and Akitake, 1975*b*, Table 2, p. 95. Reprinted by permission of the publisher.

accumulated levels of 35 mg/kg in the liver, 0.5 mg/kg in the muscle, and 9 mg/kg in the digestive tract in 4 days. Fish were then placed in clean water and much of the pentachlorophenol was rapidly eliminated. After 16 days in clean water, residues were 0.6 mg/kg in the liver, 0.02 mg/kg in the muscle, and 0.13 mg/kg in the digestive tract; control fish samples contained less than 0.01 mg/kg.

Thus, it is apparent that there are differences among fish species in their abilities to concentrate pentachlorophenol; also, residue levels vary for different tissues. In considering food consumption by humans, pentachlorophenol levels are lower in muscle than in viscera.

D.5.2.1.3 Biotransformation — Kobayashi, Akitake, and Tomiyama (1970*a*) found that the concentration of pentachlorophenol in seawater decreased during the culture of short-necked clams (*Tapes philippinarum*). Because this decrease in pentachlorophenol concentration was not accompanied by a quantitative increase in the pentachlorophenol content of the shellfish, culture water was studied intensively to determine whether pentachlorophenol was present in an altered form. This investigation showed that the decrease in pentachlorophenol concentration in the medium was not due to decomposition but rather to transformation to a bound form of pentachlorophenol, which served as a detoxification mechanism in the shellfish. The excreta of the shellfish and microorganisms in the medium had no role in the formation of bound pentachlorophenol. Figure D.5.4 shows the time course of pentachlorophenol accumulation in the clam as well as the accompanying buildup of bound pentachlorophenol in the culture medium. It can be seen that detoxification and excretion of pentachlorophenol are extremely rapid processes. Fifty hours following the beginning of the culture period, more than 80% of the pentachlorophenol found in the medium was in a detoxified, bound form.

The pentachlorophenol detoxification product was characterized by Kobayashi, Akitake, and Tomiyama (1970*b*). Following culture of the short-necked clam, they eluted the medium with ammonium acetone and analyzed the eluate by silica-gel thin-layer chromatography. The isolated conjugate displayed properties (with respect to chromatographic findings and extractability with xylene) which were identical to those of synthesized pentachlorophenol sulfate. The molar ratio of pentachlorophenol to sulfate was about 1, which indicated that the isolated conjugate was a sulfate ester of pentachlorophenol. These investigators concluded that the shellfish detoxifies pentachlorophenol largely by formation of a sulfate conjugate.

When goldfish were cultured in freshwater containing pentachlorophenol, the pattern was very similar to that seen with the short-necked clam (Akitake and Kobayashi, 1975). The amount of free pentachlorophenol in the medium decreased without a quantitatively equal increase in pentachlorophenol in the fish tissue. The goldfish were exposed until they accumulated 24.8 μg pentachlorophenol per gram body weight. The fish were transferred to pentachlorophenol-free water, and the amounts of pentachlorophenol (free and bound forms) in the fish and the water were determined (Figure D.5.5). Pentachlorophenol was excreted from the fish

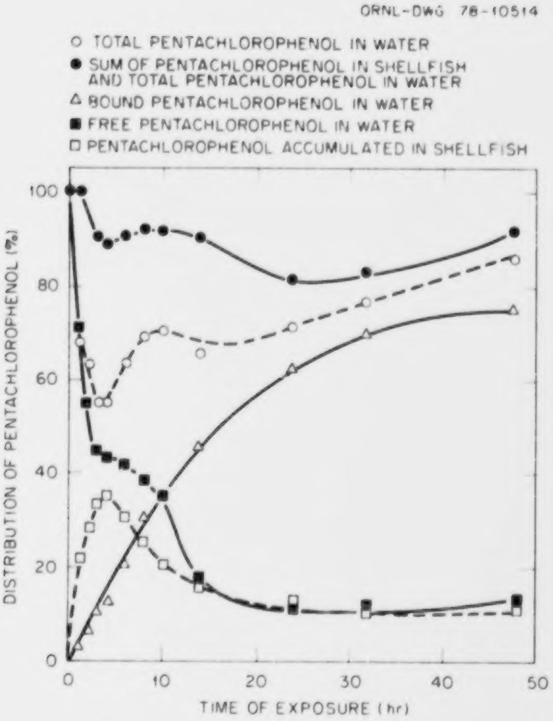

Figure D.5.4. Change with time of culture in amounts of free and bound pentachlorophenol in the medium and of pentachlorophenol in *Tapes philippinarum* from the medium. Source: Adapted from Kobayashi, Akitake, and Tomiyama, 1970*a*, Figure 3, p. 101. Reprinted by permission of the publisher.

in a conjugated form, which was determined to be pentachlorophenol sulfate. This conjugate was identical to that found in the short-necked clam by Kobayashi, Akitake, and Tomiyama (1970*b*). Apparently, the primary route of pentachlorophenol elimination in at least two aquatic organisms is conjugation to a sulfate. It is unknown how widespread this detoxification method is because other studies of pentachlorophenol detoxification in aquatic organisms did not identify degradation products.

Biodegradability and environmental fate of pentachlorophenol were studied by Lu and Metcalf (1975) in a model aquatic ecosystem which incorporated a six-element food chain that included plankton, green filamentous algae (*Oedogonium cardiacum*), snails (*Physa* sp.), water fleas (*Daphnia magna*), mosquito larvae (*Culex quinquifasciatus*), and mosquito fish (*Gambusia affinis*). The organisms were cultured in sealed 3-liter flasks. On the first day of the experiment 300 daphnia, 200 mosquito larvae, 6 snails, strands of algae, and miscellaneous plankton were placed in flasks and acclimated in a programmed environmental growth chamber for one day. Carbon-14—labeled pentachlorophenol was added to

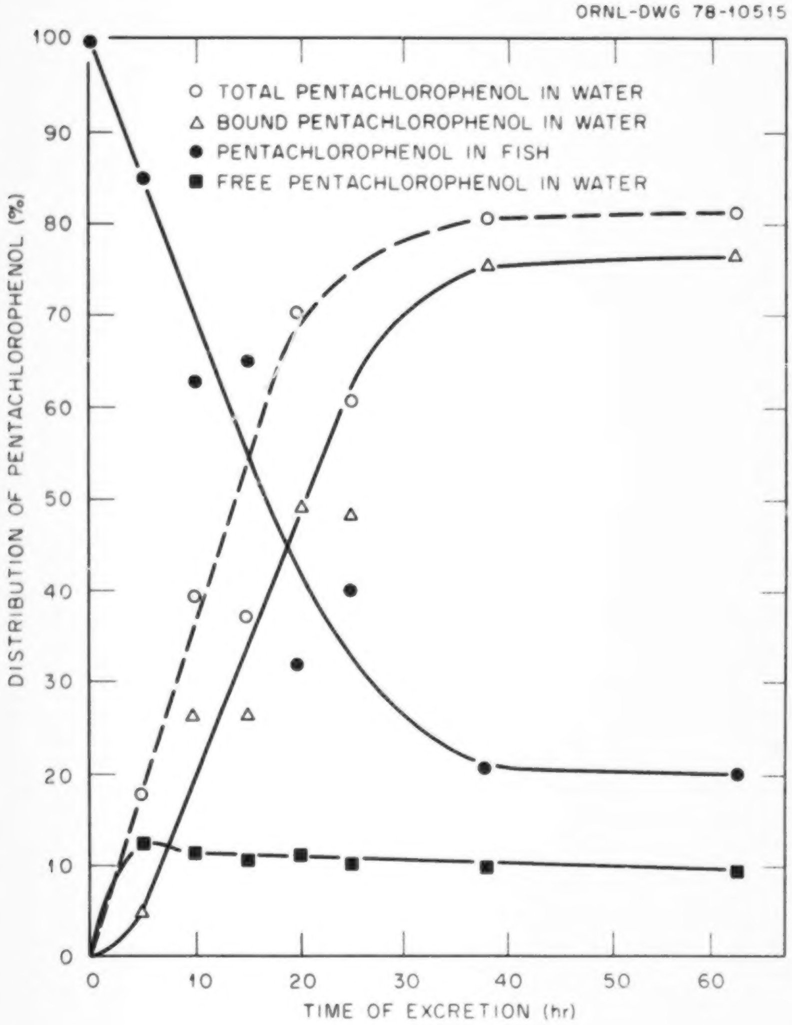

Figure D.5.5. Change in amounts of pentachlorophenol in goldfish and in free and bound pentachlorophenol in pentachlorophenol-free water. Source: Adapted from Akitake and Kobayashi, 1975, Figure 1, p. 324. Reprinted by permission of the publisher.

the flasks at a concentration of 0.01 to 0.1 mg/liter. After 24 hr, 100 daphnia and 50 mosquito larvae were removed from the flask and the [^{14}C]-pentachlorophenol content was determined. Concomitantly, 3 fish were added to the chambers. After 24 hr of incubation the experiment was terminated, and samples were concentrated and analyzed by thin-layer chromatography and autoradiography to identify pentachlorophenol and labeled metabolites (Table D.5.17). A biodegradability index, the ratio of concentrations of polar products in a given organism to nonpolar products in the same organism, was calculated for pentachlorophenol and 11

TABLE D.5.17. DISTRIBUTION OF PENTACHLOROPHENOL AND DEGRADATION PRODUCTS IN A MODEL AQUATIC ECOSYSTEM

Compound	R_f[a]	Pentachlorophenol equivalent (ng/g)					
		Culture water	Alga (*Oedogonium cardiacum*)	Daphnia (*Daphnia magna*)	Mosquito (*Culex quinquefasciatus*)	Snail (*Physa* sp.)	Fish (*Gambusia affinis*)
Total ^{14}C		7.94	10.7	1173	173	1138	1076
Unknown 1	0.75	0.21					
Pentachlorophenol	0.41	2.71	4.3	448	45.6	329	804
Unknown 2	0.34	0.38					
Unknown 3	0.26					27.2	
Unknown 4	0.22	0.04				33.2	
Unknown 5	0.16	0.09				163	
Polar	0.0	4.26	6.4	724	128	586	272
Unextractable		0.25					

[a] Retardation factor determined by thin-layer chromatography in a solvent consisting of *n*-hexane, acetone, acetic acid in ratio of 80:20:2 (v/v).

Source: Adapted from Lu and Metcalf, 1975, Table 9, p. 277. Reprinted by permission of the publisher.

other commonly used organic compounds. Values determined for the mosquito fish are given in Table D.5.18. Pentachlorophenol had relatively high biodegradability indexes: snail, 1.06; alga, 1.48; daphnia, 1.61; mosquito larvae, 2.80; and mosquito fish, 0.338. The relative detoxification capacities of these organisms are shown in Figure D.5.6. The most important means of pentachlorophenol degradation was conjugation at the phenolic OH group. Conjugation of pentachlorophenol was a highly efficient process in all organisms except the mosquito fish, which stored large amounts of unmodified pentachlorophenol over the time span of the experiment. Thus, the hazard to aquatic flora and fauna posed by pentachlorophenol appears to be intermediate among a series of commonly used organic chemicals. A true assessment of the environmental significance of pentachlorophenol in aquatic communities awaits further investigation.

D.5.2.1.4 Elimination — Kobayashi and Akitake (1975*a*) studied the kinetics of pentachlorophenol excretion in the goldfish. When goldfish were exposed to 0.1 and 0.2 mg/liter pentachlorophenol in the media for 24 hr (reaching mean tissue concentrations of 23.5 and 41.6 µg pentachlorophenol per gram body weight respectively) and were then transferred to running water, concentrations of pentachlorophenol in the fish decreased as shown in Figure D.5.7. At least two rates apparently apply to excretion of pentachlorophenol by goldfish. Excretion was initially rapid, resulting in pentachlorophenol concentrations in the fish of 20% of initial values after 20 hr. Subsequent decreases were much slower. After 100 hr, 5% of the original pentachlorophenol content was still present in the fish. Exposure time had no effect on the retention of pentachlorophenol in fish (Figure D.5.8). Fish were exposed to 0.2 mg/liter pentachlorophenol for 6, 12, 24, and 48 hr. After fish were transferred to running water, the retention curves showed similar

TABLE D.5.18. BIODEGRADABILITY INDEXES OF ORGANIC CHEMICALS DETERMINED FOR MOSQUITO FISH IN A MODEL AQUATIC ECOSYSTEM

Compound	Biodegradability index[a]
Aldrin	0.015
Aniline	1.784
Anisole	0.250
Benzoic acid	2.965
Chlorobenzene	0.014
DDT	0.012
2,6-Diethylaniline	0.139
Hexachlorobenzene	0.377
Nitrobenzene	0.023
Pentachlorophenol	0.338
Phthalic anhydride	11.884
3,5,6-Trichloro-2-pyridinol	0.311

[a] Ratio of concentrations of polar products to nonpolar products in the mosquito fish.

Source: Adapted from Lu and Metcalf, 1975, Table 1, p. 270. Reprinted by permission of the publisher.

patterns. Again, a relatively rapid initial rate of excretion was followed by a much slower rate.

The studies of Kobayashi and Akitake (1975*a*), like the investigations of Holmberg et al. (1972) (Section D.5.2.1.2), can lead to false conclusions because of deficiencies in methodologies. Neither of these studies was designed to provide reliable information on long-term residue kinetics. Kobayashi and Akitake (1975*a*) found a concentration factor of 1000-fold for a 120-hr exposure. The water was not analyzed for pentachlorophenol, and control fish were not placed in the untreated water and analyzed for pentachlorophenol residues. A few micrograms per liter of pentachlorophenol in the untreated water would contribute to the tissue levels observed at the end of the 100-hr elimination phase.

In the short-necked clam, pentachlorophenol is probably excreted through the bojanus' organ (Kobayashi, Akitake, and Tomiyama, 1969), although this assumption has not been tested rigorously. Tissue distribution studies by Kobayashi and Akitake (1975*b*) suggest that the hepatopancreas and gallbladder play important roles in the excretion of pentachlorophenol from the goldfish. Pentachlorophenol taken up by the fish is likely transferred to the hepatopancreas, detoxified, and partly transferred to the gallbladder and bile. The slow excretion kinetics

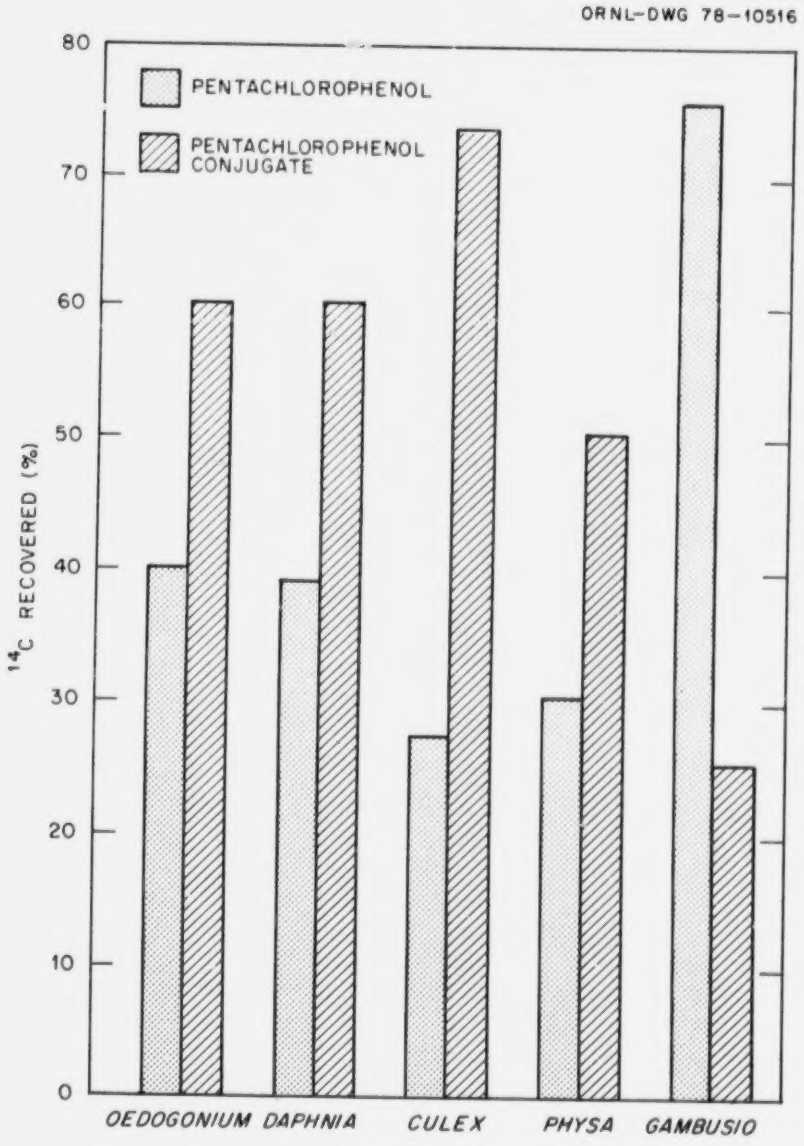

Figure D.5.6. Relative detoxification capacities of key organisms in a model aquatic ecosystem following treatment with radioactive pentachlorophenol. Source: Adapted from Lu and Metcalf, 1975, Figure 9, p. 279. Reprinted by permission of the publisher.

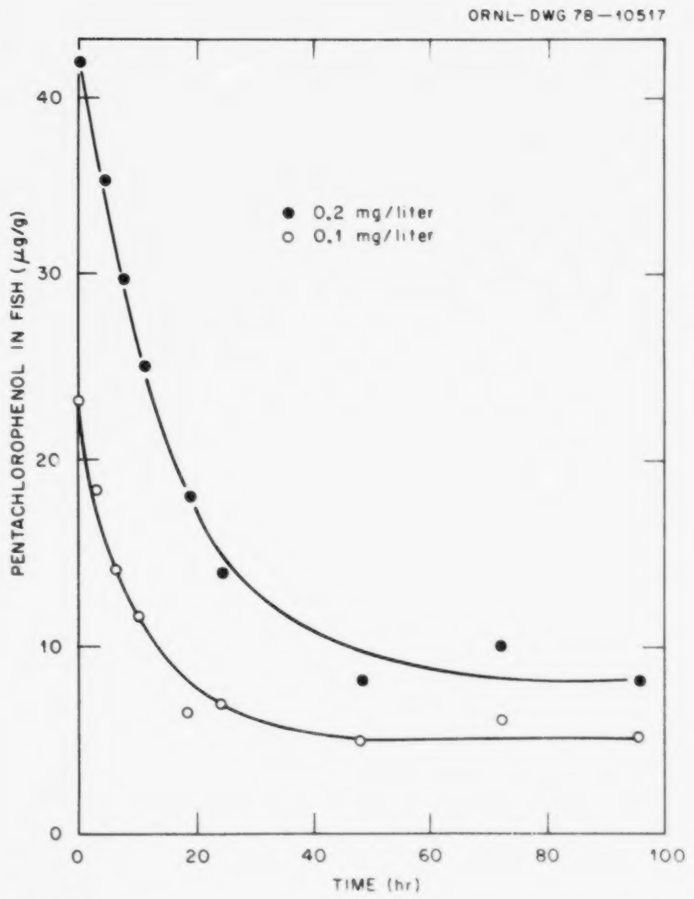

Figure D.5.7. Retention of pentachlorophenol in goldfish during culture in running water after a 24-hr exposure to pentachlorophenol media (0.1 and 0.2 mg/liter). Source: Adapted from Kobayashi and Akitake, 1975*a*, Figure 2, p. 90. Reprinted by permission of the publisher.

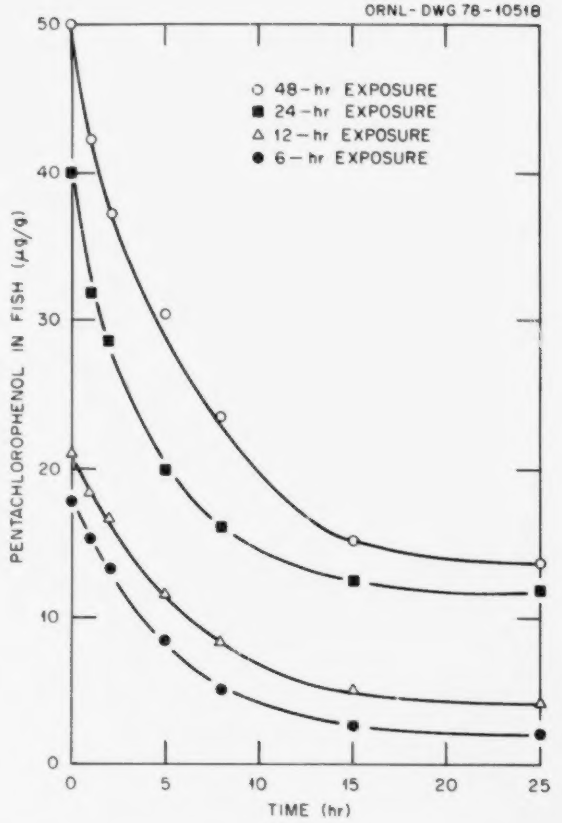

Figure D.5.8. Retention of pentachlorophenol in goldfish during culture in running water after various exposures (6 to 48 hr) to 0.2 mg/liter pentachlorophenol. Source: Adapted from Kobayashi and Akitake, 1975*a*, Figure 3, p. 90. Reprinted by permission of the publisher.

noted in the goldfish after the pentachlorophenol content reached 20% of the initial value may have resulted from storage of pentachlorophenol in the gallbladder. Other investigators have suggested that the gallbladder has an important role in the excretion of pentachlorophenol in mammals (Jakobsen and Yllner, 1971). Pentachlorophenol may also be stored in lipids, which have been suggested as a source of pentachlorophenol redistribution (Holmberg et al., 1972).

D.5.2.2 Effects

D.5.2.2.1 Physiological and Biochemical Role — There is no evidence that pentachlorophenol has a necessary metabolic role in aquatic organisms; thus, its presence in organisms must result from environmental contamination.

D.5.2.2.2 Toxicity

D.5.2.2.2.1 Mechanism of action — The biochemical mechanism of pentachlorophenol toxicity to aquatic organisms likely involves its ability to uncouple oxidative phosphorylation and at higher concentrations to inactivate glycolytic enzymes. These effects, as they relate to human toxicity, are discussed in Section D.6.2.2.1. The mechanism of pentachlorophenol toxicity to mammals probably also applies to aquatic organisms, but no direct data on this subject are available.

D.5.2.2.2.2 Local and systemic pathology — No information on local or systemic pathology in aquatic organisms was found.

D.5.2.2.2.3 Acute toxicity — Pentachlorophenol and sodium pentachlorophenate are extremely toxic to aquatic organisms when substantial amounts reach the receiving water. Numerous fish kills have resulted from seepage of pentachlorophenol-contaminated effluents or from spillage of the concentrated compound into waterways. The use of pentachlorophenol (or sodium pentachlorophenate) as a herbicide also has caused death of aquatic organisms. Shim and Self (1973) reported that the use of sodium pentachlorophenate as a herbicide in Korean rice fields caused 100% mortality of larvivorous fish. The 100% mortality occurred at dosages as little as 10% of concentrations prevalently used for herbicidal purposes. Significant damage to soft clams in tidelands around the Aniake Bay of Japan was observed by Nitta (1972). Widespread death of fish occurred in Lake Biwa, Japan, following a heavy rain immediately after herbicidal application of pentachlorophenol to paddy fields around the Chikugo River.

The susceptibility of different fish species to pentachlorophenol toxicity varies. Of 19 species of fish studied by Goodnight (1942), the most sensitive species were unable to survive at pentachlorophenol levels greater than 0.2 mg/liter; more tolerant species survived at 0.4 to 0.6 mg/liter. However, aquatic invertebrates are more tolerant than fish to the effects of pentachlorophenol and are able to survive at concentrations which kill many species of fish. Acute toxic responses of fish and aquatic invertebrates to pentachlorophenol are compiled in Table D.5.19. Minimum lethal concentrations of sodium pentachlorophenate for

TABLE D.5.19. ACUTE TOXICITY OF PENTACHLOROPHENOL TO AQUATIC ORGANISMS

Species	Time (hr)	Pentachlorophenol concentration (μg/liter)	Source
		Levels causing 50% lethality	
Bluegill (*Lepomis macrochirus*)	48	29-39	Inglis and Davis, 1973
	24	350	Turnbull, DeMann, and Weston, 1954
Guppy (*Poecilia reticulata*)	96	250	Anderson and Weber, 1975
Guppy (*Lebistes reticulatus*)	6	4,000	Klock, 1956
Fish (*Aplocheilus latipes*)	24	140	Shim and Self, 1973
Fish (*Zacco platypus*)	24	230	Shim and Self, 1973
"Guchi" fish (*Nibea albiflora*)	48	80	Tomiyama and Kawabe, 1962
"Warasubo" fish (*Odontamblyopus rubicundus*)	48	250	Tomiyama and Kawabe, 1962
Eel (*Anguilla japonica*)	24	200	Tomiyama and Kawabe, 1962
Rainbow trout (*Salmo gairdneri*)	96	47-110	Davis and Hoos, 1975
Coho salmon (*Oncorhynchus kisutch*)	96	32-93	Davis and Hoos, 1975
	24	150	Hanes et al., 1968
Sockeye salmon (*Oncorhynchus nerka*)	96	50-130	Davis and Hoos, 1975
Underyearling	96	63	Webb and Brett, 1973
Fathead minnow (*Pimephales promelas*)	96	210-340	Ruesink and Smith, 1975
	96	210	Adelman, Smith, and Siesennop, 1976
Goldfish (*Carassius auratus*)	12	200	Kobayashi and Akitake, 1975*a*
	96	49-56	Inglis and Davis, 1973
Channel catfish (*Ictalurus punctatus*)	48	460	Clemens and Sneed, 1959
Juvenile Atlantic salmon (*Salmo salar*)	96	60	Coté, 1972
Short-necked clam (*Tapes philippinarum*)	120	200	Kobayashi, Kurokawa, and Tomiyama, 1969
Tubifex worms (*Tubifex tubifex* and *Limnodrilus hoffmeisteri*)	24	310-1,400	Whitley, 1967
"Shiraebi" shrimp (*Leander japonicus*)	48	2,300	Tomiyama and Kawabe, 1962
Mosquito larvae (*Culex tritaeniorhynchus*)	24	20,900	Shim and Self, 1973
White crappie (*Pomoxis annularis*)		65-75[a]	Springer, 1957
		Levels causing 100% lethality	
Steelhead trout (*Salmo gairdneri*)			
Embryos		50	Chapman, 1969
Alevins		200	Chapman, 1969
Oyster eggs and larvae (*Crassostrea virginica*)		250	Davis, 1961
Clam eggs and larvae (*Venus mercenaria*)		250	Davis, 1961
Shellfish (*Venerupis philippinarum*)		100	Tomiyama et al., 1962

(continued)

TABLE D.5.19 (continued)

Species	Time (hr)	Pentachlorophenol concentration (μg/liter)	Source
Levels producing no toxic effect			
Shellfish (*Conchocelis* sp.)		800	Tomiyama, Kobayashi, and Kawabe, 1962[a]
Crayfish (*Carbarus virilis*)		9,500	Springer, 1957
		5,000	Goodnight, 1942
Amphipod (*Hyalella knickerbockeri*)		5,000	Goodnight, 1942
Cladocera (*Daphnia pulex*)		5,000	Goodnight, 1942
Dragonfly nymphs (*Epicordulia* sp.)		5,000	Goodnight, 1942
Damsel fly nymphs (*Ischnura* sp.)		5,000	Goodnight, 1942
Isopod (*Asellus communis*)		5,000	Goodnight, 1942
Levels ranging between 100% survival and 100% lethality			
Silver-mouthed minnow (*Ericymba buccata*)		200-400	Goodnight, 1942
Blackfin minnow (*Notropis umbratilis*)	6.3	400-600	Goodnight, 1942
Doughbelly (*Campostoma anomalum*)	4.3	200-400	Goodnight, 1942
Steel-colored minnow (*Notropis whipplii*)	5.3	200-400	Goodnight, 1942
Blunt-nosed minnow (*Pimephales notatus*)	7.5	200-400	Goodnight, 1942
Horned dace (*Semotilus atromaculatus*)	6.3	400-600	Goodnight, 1942
Orange-spotted sunfish (*Lepomis humilis*)	6.8	200-400	Goodnight, 1942
Top minnow (*Fundulus notatus*)	9.8	600-800	Goodnight, 1942
Tadpole (*Rana pipiens*)	9.5	600-800	Goodnight, 1942

[a] Minimum lethal concentration.

fish and aquatic invertebrates as reported by Norup (1972) are shown in Figure D.5.9. This graph illustrates two points: (1) a considerable range of minimum lethal concentrations exists for various species of fish, and (2) although some studies have reported that invertebrates are more tolerant than fish to sodium pentachlorophenate, many invertebrates apparently tend to be just as sensitive.

Chapman (1969) found that sodium pentachlorophenate was lethal to trout alevins at a concentration of 70 μg/liter and to embryos at 50 μg/liter. Additionally, 40 μg/liter was invariably lethal if the embryo and alevin stages were exposed to sodium pentachlorophenate. Growth of the alevin was reduced by approximately 6% for each 10 μg/liter increment. Reduced growth rates, reduced efficiency of yolk utilization, and increased rates of yolk metabolism and oxygen consumption were evident. Thus, relatively low concentrations of sodium pentachlorophenate caused adverse effects.

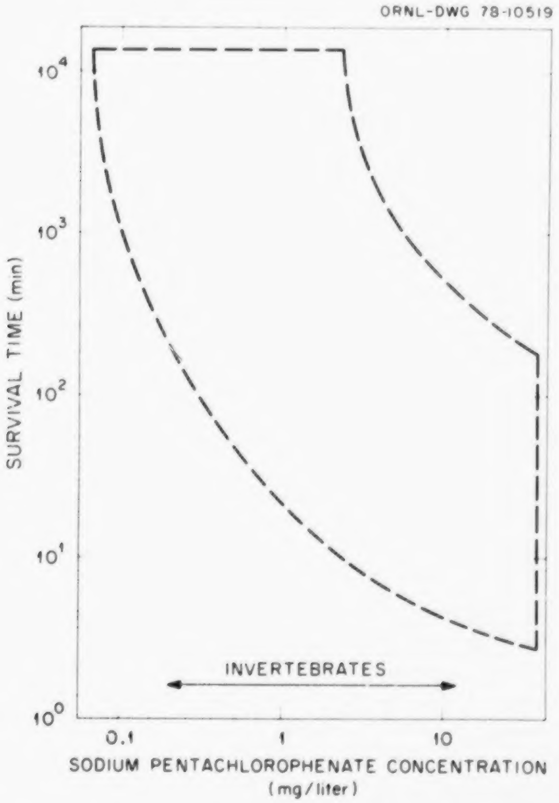

Figure D.5.9. Boundary of lethal area for a variety of fish species, relating lethal concentration of sodium pentachlorophenate to time of survival. The lethal concentrations for invertebrates are similar to those for fish. Source: Adapted from Norup, 1972, Figure 1, p. 1586. Reprinted by permission of the publisher.

Danil'chenko and Stroganov (1975) studied the effect of sodium pentachlorophenate on the embryonic and early postembryonic development of freshwater fish. Fertilized fish eggs were cultured in Moscow river water containing pentachlorophenol. The number of embryo deaths and developmental abnormalities were noted for pike (*Esox lucius*), perch (*Perca fluviatilis*), ruffe (*Acerina cernua*), roach (*Rutilus rutilus*), minnow (*Phoxinus phoxinus*), gudgeon (*Gobio gobio*), loach (*Misgurnus fossilis*), and spiny loach (*Cobitis taenia*). Death of embryos and morphological disturbances, such as dropsy of the pericardium, elongation of the heart chambers, and absence or retardation of inflation of the swim bladder, were seen in all species tested when the sodium pentachlorophenate concentration in the culture medium was greater than 0.5 mg/liter. The effect of sodium pentachlorophenate on embryonic development depended on the species. The most resistant species were pike, perch, and ruffe; embryos of the spiny loach, minnow, and roach were the least resistant. The specific kinds of morphological alterations also varied among the species. At 1 mg/liter sodium pentachlorophenate development ceased following overgrowth of the vitellus of the blastoderm in the perch, ruffe, pike, and loach. Development was arrested at the morula stage in the minnow and the spiny loach. There were no morphological disturbances or deaths when fish eggs were cultured in media containing 0.1 mg/liter pentachlorophenol. These investigators concluded that the maximum permissible concentration of sodium pentachlorophenate in water should be 0.1 mg/liter. Small but significant deviations from the control, such as length of prolarvae and larvae, time of hatching, and heart rate, were noted in fish embryos cultured at lower concentrations of pentachlorophenol (0.01 and 0.001 mg/liter). Further study is needed to verify these findings and to determine the mechanism of toxic action.

In an effort to control schistosomiasis, a severe endemic disease of humans in much of Asia, Africa, and South America, sodium pentachlorophenate has been used to destroy *Australorbis glabratus*, the snail which serves as intermediate host for schistosomes. This compound is extremely effective and is probably the most widely used molluscicide. At a concentration of 10 mg/liter, sodium pentachlorophenate was lethal to 90% to 100% of the snails (Berry, Nolan, and González, 1950). Adult snails placed in sodium pentachlorophenate solutions containing 0.05 and 0.1 mg/liter for seven to eight days generally showed reduced egg production. Removal of the chemical from the snails' environment resulted in egg production and improved egg viability (Olivier and Haskins, 1960). Yasuraoka and Hosaka (1971) tested the toxicity of sodium pentachlorophenate to the snail *Oncomelania nosophora*. Snails collected from villages in the Yamanashi area of Japan were tested yearly for susceptibility to sodium pentachlorophenate toxicity. The LC_{50} values over a ten-year period ranged from 0.25 to 0.37 mg/liter.

Shiff and Garnett (1961) studied the effect of molluscicidal concentrations of pentachlorophenol on the microflora and microfauna of small, biologically stable fish ponds in Southern Rhodesia. The aquatic organisms present included Cladocera, aquatic insect larvae, Copepoda, *Copepod nauplii*, Ostracoda, and *Spirogyra* sp. Ponds (4 by 4 m) were treated with sodium pentachlorophenate until the level reached 5 mg/liter. Total numbers of microflora and microfauna and fluctuations among groups of microflora and microfauna in the pond were determined. A dramatic reduction in all forms of planktonic life was seen immediately after sodium pentachlorophenate was added. The effect, however, was generally short-lived; 32 days after application population trends returned to normal.

Tagatz et al. (1977) studied the effects of pentachlorophenol on estuarine organisms during a nine-week exposure. Populations of mollusks decreased at pentachlorophenol levels of 7 μg/liter, and the number of annelids and arthropods decreased at 76 μg/liter. Almost no animals survived at 622 μg/liter. Pentachlorophenol was not found in the aquaria sediment in the 7 μg/liter tank. In the 76 and 622 μg/liter tanks, 0.7 to 9 μg/kg pentachlorophenol was recovered from the sediment.

The eight-day LC_{50} for crayfish (*Astacus fluviatilis*) exposed to pentachlorophenol was determined by Kaila and Saarikoski (1977). At pH 7.5 the LC_{50} was 53 mg/liter, and at pH 6.5 it was 9.0 mg/liter.

D.5.2.2.2.4 Chronic toxicity — The lethal concentrations in Table D.5.19 are a poor indication of the true environmental significance of pentachlorophenol. Dramatic effects such as massive fish kills are readily seen and are aesthetically objectionable; however, other more subtle effects may occur in aquatic communities exposed to pentachlorophenol residues. This exposure may decrease the ability of some species to reproduce, thereby affecting the survival of a species or even the survival and integrity of entire biological communities. The chronic effects of pentachlorophenol on aquatic organisms are summarized in Table D.5.20. Decreased growth and a curtailment of food conversion efficiency have been reported. Serious effects such as renal degeneration, liver damage, and retarded gonadal development were found by Crandall and Goodnight (1963).

The metabolic effects of pentachlorophenol on the eel (*Anguilla anguilla* L.) have been examined by Holmberg et al. (1972). Eels were exposed to 0.1 mg/liter pentachlorophenol in seawater and freshwater. In the seawater tests, the eels were exposed for 8 days to pentachlorophenol-containing seawater followed by 8 days in pentachlorophenol-free water. The freshwater tests involved exposure to pentachlorophenol-containing water for 4 days followed by a recovery period of 55 days. The effects of these exposures on a large number of metabolic parameters are shown in Tables D.5.21 and D.5.22. The notable effects are summarized in Table D.5.23. The pentachlorophenol-treated eels did not fully recover despite spending two months in clean water. A schematic diagram of the action of pentachlorophenol on eels was prepared by Larsson (1973) and is presented as Figure D.5.10.

Bostrom and Johansson (1972) studied the effect of sublethal concentrations of pentachlorophenol on liver enzymes such as hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, pyruvate kinase, lactate dehydrogenase, fumarase, and cytochrome oxidase

TABLE D.5.20. CHRONIC EFFECTS OF PENTACHLOROPHENOL ON AQUATIC ORGANISMS

Species	Time (days)	Pentachlorophenol concentration (μg/liter)	Experimental conditions	Effects	Source
Coho salmon (*Oncorhynchus kisutch*)	14	100	Potassium pentachlorophenate; 11°C with 12-hr dark and light periods in filtered spring water	Fatty acid catabolism at 47% of available acids (25% for controls); catabolism proportional to available mass of individual fatty acids	Hanes et al., 1968
Guppy (*Lebistes reticulatus*)	180	500	Sodium pentachlorophenate; total hardness 165 mg/liter; total alkalinity 99 mg/liter; dissolved oxygen 8.5 mg/liter; 12-hr dark and light periods; pH 8.4 to 8.6	Growth inhibition; lack of mesenteric fat; dilation of renal tubules with degeneration of tubular epithelial cells; retarded gonadal development; liver damage	Crandall and Goodnight, 1963
Cichlid fish	10	200	Potassium pentachlorophenate; 25°C	Increased caloric loss during starvation; increased food intake and energy losses; decreased growth; caloric cost of exercise increased relative to controls	Krueger et al., 1968
Tubifex worms (*Tubifex tubifex* and *Limnodrilus hoffmeisteri*)	<1	100-1250	Sodium pentachlorophenate; Knopps solution 0.01% at pH 9.5	Increasing respiration rate (expressed as oxygen consumed per milligam per hour) with increasing sodium pentachlorophenate levels	Whitley and Sikora, 1970
Underyearling sockeye salmon (*Oncorhynchus nerka*)	14-56	0-50	Sodium pentachlorophenate; dissolved oxygen at 9% to 100% saturation; pH 6.8; 16-hr photoperiod	Decreased growth (threshold concentration = 1.74 μg/liter); decreased food conversion (threshold concentration = 1.8 μg/liter); no effect on swimming performance	Webb and Brett, 1973
Pike (*Esox lucius*), perch (*Perca fluviatilis*), ruffe (*Acerina cernua*), roach (*Rutilis rutilis*), minnow (*Phoxinus phoxinus*), gudgeon (*Gobio gobio*), loach (*Misgurnus fossilis*), and spiney loach (*Cobitis taenia*)		1-5000	Sodium pentachlorophenate; Moscow river water; embryo development monitored	Severe developmental abnormalities in all species at concentrations >1 mg/liter	Danil'chenko and Stroganov, 1975
Shellfish (*Venerupis philippinarum*)	1	100	Seawater	Appreciable decrease in ^{32}P uptake by the shellfish relative to control animals	Tomiyama, Kobayashi, and Kawabe, 1962b

TABLE D.5.21. METABOLIC EFFECTS OF PENTACHLOROPHENOL (0.1 mg/liter) ON EELS IN FRESHWATER

Parameter	4-day exposure		4-day exposure + 4-day recovery		4-day exposure + 55-day recovery
	Control[a]	Treated[b]	Control[a]	Treated[b]	Treated[a]
Decrease in body weight, %	2.4 ± 1.0	6.0 ± 1.3[c]	3.9 ± 0.8	7.9 ± 1.5[c]	16.2 ± 1.8
Hematocrit, %	28.3 ± 1.5	37.7 ± 2.3[d]	31.0 ± 1.6	37.5 ± 0.7[d]	35.7 ± 1.8
Hemoglobin, mg/100 ml	9.2 ± 0.3	11.1 ± 0.4[d]	10.1 ± 0.4	10.6 ± 0.5	9.4 ± 0.6
Plasma chloride, mM	114 ± 3	111 ± 1	113 ± 3	103 ± 4	96 ± 4
Plasma inorganic phosphate, mg/100 ml	7.0 ± 0.7	10.13 ± 1.3[c]	6.9 ± 0.6	7.0 ± 0.3	5.4 ± 0.3
Total plasma protein, g/100 ml	5.1 ± 0.4	5.2 ± 0.2	5.3 ± 0.2	5.1 ± 0.2	5.2 ± 0.3
Total plasma cholesterol, mg/100 ml	252 ± 23	339 ± 16[c]	270 ± 18	304 ± 17	306 ± 22
Esterified plasma cholesterol, mg/100 ml	166 ± 12	225 ± 10[d]	174 ± 13	195 ± 13	201 ± 19
Free plasma cholesterol, mg/100 ml	86 ± 14	111 ± 14	99 ± 7	109 ± 12	105 ± 9
Plasma triglycerides, mM	3.5 ± 0.7	4.0 ± 0.9	4.1 ± 0.7	4.8 ± 1.5	4.5 ± 0.6
Plasma free fatty acids, μM	459 ± 43	517 ± 33	437 ± 34	513 ± 37	552 ± 42
Blood glucose, mg/100 ml	53.8 ± 6.7	152 ± 18[d]	68 ± 15	124 ± 20.1[c]	109 ± 29
Blood lactate, mg/100 ml	11.5 ± 2.2	16.7 ± 2.8	7.1 ± 0.6	6.9 ± 1.3	12.3 ± 1.6
Liver-somatic index, (liver wt x 100)/body wt	1.2 ± 0.1	1.23 ± 0.08	1.3 ± 0.06	1.26 ± 0.08	1.51 ± 0.05
Liver triglycerides, mg/100 mg wet wt	5.1 ± 1.4	4.3 ± 0.8	3.4 ± 1.7	3.2 ± 1.3	3.1 ± 1.2
Muscle triglycerides, mg/100 mg wet wt	9.1 ± 1.3	6.5 ± 1.1	7.1 ± 0.4	5.5 ± 0.7	4.9 ± 0.6
Muscle glycogen, mg/100 mg wet wt	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.12 ± 0.03	0.24 ± 0.02

[a] Eight eels in group.
[b] Nine eels in group.
[c] $P < 0.05$.
[d] $P < 0.005$.

Source: Adapted from Holmberg et al., 1972, Table 3, p. 180. Reprinted by permission of the publisher.

TABLE D.5.22. METABOLIC EFFECTS OF PENTACHLOROPHENOL (0.1 mg/liter) ON EELS IN SEAWATER

Parameter	8-day exposure		8-day exposure + 8-day recovery	
	Control[a]	Treated[b]	Control[a]	Treated[b]
Decrease in body weight, %	1.3 ± 0.5	1.7 ± 0.6	2.2 ± 0.7	6.2 ± 0.8[d]
Hematocrit, %	38.4 ± 0.5	41.1 ± 0.8[c]	35.3 ± 0.4	38.3 ± 0.3[d]
Hemoglobin, mg/100 ml	10.5 ± 0.5	12.3 ± 0.5[c]	10.0 ± 0.3	11.6 ± 0.4[d]
Plasma chloride, mM	141 ± 5	141 ± 3	144 ± 2	145 ± 1
Plasma inorganic phosphate, mg/100 ml	7.2 ± 0.4	8.3 ± 0.3[c]	7.0 ± 0.2	7.1 ± 0.2
Total plasma protein, g/100 ml	4.0 ± 0.1	4.5 ± 0.1[d]	4.4 ± 0.2	4.6 ± 0.2
Total plasma cholesterol, mg/100 ml	373 ± 40	378 ± 39	336 ± 22	433 ± 31[c]
Esterified plasma cholesterol, mg/100 ml	227 ± 23	253 ± 19	234 ± 19	260 ± 24
Free plasma cholesterol, mg/100 ml	147 ± 27	125 ± 23	101 ± 15	172 ± 11[d]
Plasma triglycerides, mM	3.6 ± 0.9	6.2 ± 0.8[c]	3.7 ± 0.4	5.0 ± 0.7
Plasma free fatty acids, μM	399 ± 16	424 ± 17	369 ± 13	565 ± 51[d]
Blood glucose, mg/100 ml	45.1 ± 2.7	116.3 ± 5.5[d]	40.4 ± 2.1	85.7 ± 2.9[d]
Blood lactate, mg/100 ml	7.5 ± 0.8	12.0 ± 1.1[d]	7.4 ± 1.1	12.1 ± 1.3[c]
Liver-somatic index, (liver wt x 100)/body wt	1.29 ± 0.09	1.25 ± 0.11	1.21 ± 0.04	1.44 ± 0.08[c]
Liver triglycerides, mg/100 mg wet wt	6.0 ± 1.5	4.2 ± 1.5	5.4 ± 1.9	5.3 ± 1.5
Muscle triglycerides, mg/100 mg wet wt	11.3 ± 2.4	8.2 ± 1.7	9.0 ± 2.1	6.6 ± 1.4
Liver glycogen, mg/100 mg wet wt	3.2 ± 0.5	2.5 ± 0.4	3.3 ± 0.3	2.6 ± 0.6
Muscle glycogen, mg/100 mg wet wt	0.16 ± 0.01	0.11 ± 0.02[c]	0.11 ± 0.02	0.14 ± 0.03
Liver glutamate pyruvate transaminase, milliunit/mg wet wt	30.7 ± 7.6	27.3 ± 4.3	42.6 ± 6.9	24.9 ± 3.7[c]
Muscle glutamate pyruvate transaminase, milliunit/mg wet wt	0.65 ± 0.12	0.47 ± 0.07	0.59 ± 0.07	0.39 ± 0.04[c]

[a] Eight eels in group.
[b] Nine eels in group.
[c] $P < 0.05$.
[d] $P < 0.005$.

Source: Adapted from Holmberg et al., 1972, Table 4, p. 181. Reprinted by permission of the publisher.

which are involved in energy metabolism in the eel. Eels were maintained in freshwater containing 0.1 mg/liter sodium pentachlorophenate for 4 days. Following treatment, the eels recovered for 30 days in pentachlorophenol-free freshwater. Activities of pyruvate kinase and lactate dehydrogenase decreased after pentachlorophenol treatment, but activities of hexokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, fumarase, and cytochrome oxidase increased. Following the 30-day recovery period, enzyme activities approximated those of

TABLE D.5.23. EFFECTS OF PENTACHLOROPHENOL ON THE EEL

Parameter	Effect
Hematocrit	Rapid and persisting increase
Hemoglobin	Rapid and persisting increase
Inorganic phosphate	Initial increase
Blood glucose	Rapid and persisting increase
Blood lactate	Rapid increase
Plasma triglycerides	Increase
Plasma free fatty acids	Slow increase
Plasma esterified cholesterol	Slight increase
Plasma free cholesterol	Slight increase
Liver-somatic index	Slight increase
Liver glycogen	Slight initial decrease
Liver glutamate pyruvate transaminase	Decrease
Muscle glutamate pyruvate transaminase	Decrease

Source: Adapted from Larsson, 1973, Figure 2, p. 623. Reprinted by permission of the publisher.

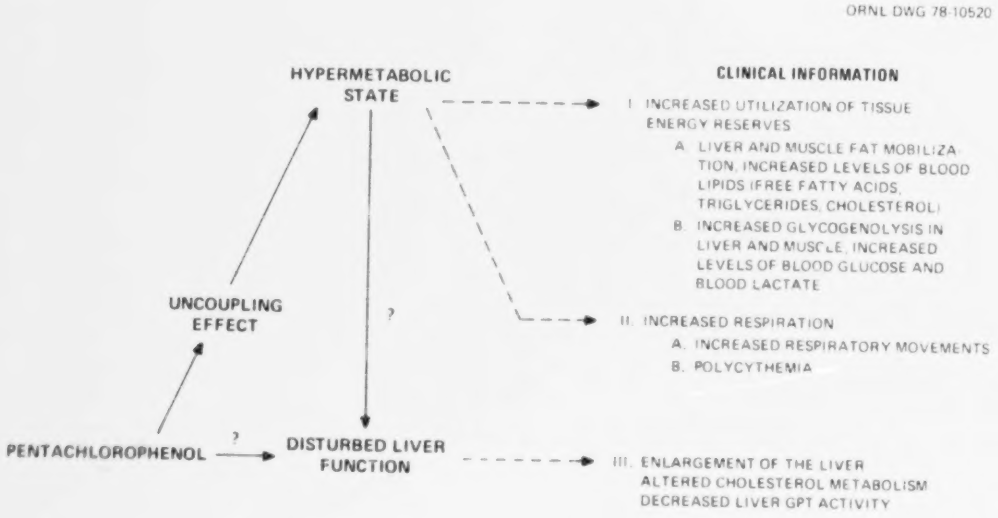
ORNL DWG 78-10520
HYPERMETABOLIC STATE
CLINICAL INFORMATION
I. INCREASED UTILIZATION OF TISSUE ENERGY RESERVES
A. LIVER AND MUSCLE FAT MOBILIZATION, INCREASED LEVELS OF BLOOD LIPIDS (FREE FATTY ACIDS, TRIGLYCERIDES, CHOLESTEROL)
B. INCREASED GLYCOGENOLYSIS IN LIVER AND MUSCLE, INCREASED LEVELS OF BLOOD GLUCOSE AND BLOOD LACTATE
UNCOUPLING EFFECT
?
II. INCREASED RESPIRATION
A. INCREASED RESPIRATORY MOVEMENTS
B. POLYCYTHEMIA
PENTACHLOROPHENOL
?
DISTURBED LIVER FUNCTION
III. ENLARGEMENT OF THE LIVER
ALTERED CHOLESTEROL METABOLISM
DECREASED LIVER GPT ACTIVITY

Figure D.5.10. Action of pentachlorophenol on eels in vivo. Source: Adapted from Larsson, 1973, Figure 3, p. 624. Reprinted by permission of the publisher.

control animals. In in vitro tests, pentachlorophenol inhibited all enzymes.

These reports by Holmberg et al. (1972) and Bostrom and Johansson (1972) indicate that the effects of pentachlorophenol at the molecular level may persist following single sublethal exposures. Pentachlorophenol remained above background levels in the eels for the duration of the experiment, and these low levels of pentachlorophenol in the tissues may exert long-term toxic effects. Additional research to determine whether enzyme systems are permanently damaged is needed.

D.5.2.2.2.5 Effect of environmental parameters on toxicity – The toxicity values in Table D.5.19 as well as those reported elsewhere should be considered estimates. In a bioassay standardization study by Davis and Hoos (1975), seven government and private laboratories in British Columbia were provided identical reference toxicants and comprehensive guidelines to be used in conducting the bioassays. Data obtained are shown in Table D.5.24. The results, expressed as 96-hr LC_{50} values, varied as much as threefold among laboratories. Thus, even when experimental conditions and the toxicant are rigidly standardized, large variations among investigators are frequently encountered. When different laboratories using different techniques and sources of toxicant perform similar experiments, data variability may be much greater than threefold.

Water conditions such as temperature, pH, and salinity may affect the toxicity of pentachlorophenol to aquatic organisms, either directly

TABLE D.5.24. SUMMARY OF RESULTS REPORTED BY SEVEN LABORATORIES USING SODIUM PENTACHLOROPHENATE AS A REFERENCE TOXICANT FOR SALMONID BIOASSAYS

Species	Laboratory code	LC_{50} (μg/liter)	
		Log-probit estimate	Nomographic calculation[a]
Rainbow trout (*Salmo gairdneri*)	A	48	47 (32.9, 67.2)
	B	100	106 (88.5, 127.0)
	C	50	50 (29.8, 84.0)
	D	96	96 (90.1, 102.2)
	E	92	98 (87.5, 109.8)
	F	75	[b]
Coho salmon (*Oncorhynchus kisutch*)	C	37	31.8 (24.9, 40.6)
	E	96	92 (79.3, 106.7)
Sockeye salmon (*Oncorhynchus nerka*)	D	50	50 (40.3, 62.0)
	G	130	130 (119, 142)

[a] 95% confidence limits; upper and lower limits in parentheses.
[b] Insufficient data for calculation of confidence limits.

Source: Adapted from Davis and Hoos, 1975, Table 2, p. 414. Reprinted by permission of the publisher.

by changing the metabolic rate of the organisms or indirectly by altering the biological availability of pentachlorophenol. Goodnight (1942) reported that temperature — in the range of 9°C to 24°C — did not markedly influence the toxicity of sodium pentachlorophenate to fish. At higher temperatures — 28°C to 30°C — fish succumbed more rapidly to sodium pentachlorophenate poisoning, but this higher fatality rate was ascribed to the high temperature per se and the resulting depleted oxygen content of the water. A pronounced effect of pH on the toxicity of sodium pentachlorophenate was noted by Goodnight (1942). The pH of the medium varied from 5 to 8; fish survived longer in pentachlorophenol-contaminated water at pH 7.6 than at pH 6.6 or less. The increased survival time, especially at low concentrations of sodium pentachlorophenate (<0.4 mg/liter), was several hours. Above the subsistence level of dissolved oxygen content (2 mg/liter for most fish), variation in oxygen content of the water did not affect the survival time of fish. Ruesink and Smith (1975) determined the 96-hr LC_{50} for fathead minnows exposed to sodium pentachlorophenate at 15°C and 25°C and found increased toxicity at the higher temperature (Table D.5.25).

TABLE D.5.25. THE 48- AND 96-hr LC_{50} VALUES AND THE LETHAL THRESHOLD CONCENTRATION OF SODIUM PENTACHLOROPHENATE FOR FATHEAD MINNOWS AT 15°C AND 25°C

Temperature (°C)	48-hr LC_{50} (mg/liter)	96-hr LC_{50} (mg/liter)	Lethal threshold concentration (mg/liter)
15	0.21	0.21	0.21
25	0.37	0.34	0.33

Source: Adapted from Ruesink and Smith, 1975, Table 2, p. 569. Reprinted by permission of the publisher.

Studies of the acute toxicity of pentachlorophenol to fish at different water hardnesses (13 to 365 mg/liter) showed that hardness had little or no effect on toxicity to bluegill (*Lepomis macrochirus*), rainbow trout (*Salmo gairdneri*), bluespotted sunfish (*Lepomis* sp.), goldfish (*Carassius auratus*), redear sunfish (*Lepomis* sp.), and black bullhead (*Ictalurus* sp.) (Inglis and Davis, 1973). Determination of the effect of pH on the toxicity of sodium pentachlorophenate to tubifex worms showed TL_m values of 0.31, 0.67, and 1.4 mg/liter at pH 7.5, 8.6, and 9.5 respectively. This significant effect confirms the findings with other aquatic animals showing that a decrease in pH enhances the toxic effect of sodium pentachlorophenate.

Alderdice (1963) studied the median resistance times of juvenile coho salmon (*Oncorhynchus kisutch*) to 3 mg/liter sodium pentachlorophenate at different combinations of salinity, temperature, and dissolved oxygen content. Each parameter affected the toxicity of sodium pentachlorophenate. Coho salmon demonstrated maximum resistance to sodium pentachlorophenate at 17.68 g/liter salinity, 4.86°C, and 7.66 mg/liter oxygen.

When the hazards associated with the presence of a toxic substance in the environment are assessed, it is important to consider the possible effects of other pollutants, either natural or man-made, on the toxicity of the compound. For example, Statham and Lech (1975) found that exposure of rainbow trout to a nonlethal concentration of carbaryl (1-naphthyl-N-methylcarbamate) significantly increased the acute toxicity of pentachlorophenol in aqueous solutions. Enhancement of toxicity by carbaryl was a general phenomenon; potentiation was also observed when the acute toxicities of 2,4-D *n*-butyl ester, dieldrin, and rotenone were examined. This phenomenon was believed to have occurred because carbaryl increased the uptake of the toxicant from the water. Other compounds present in water also likely produce alterations in the toxic effects of sodium pentachlorophenate. Furthermore, the toxicity of sodium pentachlorophenate may be decreased if appropriate substances are present in the water. The toxicity of two or more compounds in combination is worthy of additional investigation.

D.5.2.2.3 Bioconcentration and Biomagnification in Aquatic Organisms — Bioconcentration is the ability of an organism to concentrate a substance from an aquatic system. Biomagnification refers to the substance being found at successively higher concentrations with increasing trophic levels in the food chain. Many aquatic organisms are capable of pentachlorophenol bioconcentration. Pentachlorophenol may accumulate in fish in two ways: (1) following acute exposure due to tissue storage and a slow excretion rate or (2) by constant uptake during exposure to constant ambient levels of pentachlorophenol. Accumulation in the latter case results from the rate of uptake exceeding the excretion rate. Tissue levels of pentachlorophenol under these circumstances depend on the ability of the fish to excrete the compound and on the levels of pentachlorophenol in the water. Kobayashi and Akitake (1975*a*) demonstrated that pentachlorophenol accumulation in the goldfish increased when the fish were confined to culture media in which a constant level of pentachlorophenol was maintained until either the fish died or the pentachlorophenol content was at a high chronic level. As discussed in Sections D.5.2.1.2 and D.5.2.1.4, the reported studies do not provide sufficient data to draw reliable conclusions on the long-term retention of pentachlorophenol in aquatic species.

Pentachlorophenol biomagnification has not been clearly demonstrated in the environment. Biomagnification has been demonstrated, however, by Lu and Metcalf (1975) in a model aquatic ecosystem. A six-element food chain included, plankton, green filamentous algae (*Oedogonium cardiacum*), snails (*Physa* sp.), water fleas (*Daphnia magna*), mosquito larvae (*Culex quinquifasciatus*), and mosquito fish (*Gambusia affinis*). The experimental procedure used by these investigators is discussed in detail in

Section D.5.2.1.3. An ecological magnification factor (defined as ratio of concentration of pentachlorophenol in the organism to concentration of pentachlorophenol in the water) was calculated for each member of the model ecosystem. The value calculated for mosquito fish was 296; other organisms had lower values: algae, 1.58; mosquito larvae, 16; snail, 121; and daphnia, 165. The correspondence between ecological magnification factor and trophic level indicates that biomagnification likely occurred. The ecological magnification factor of 11 other commonly used organic compounds was also determined for the mosquito fish (Table D.5.26). The value for pentachlorophenol is roughly intermediate to those for other compounds tested.

TABLE D.5.26. ECOLOGICAL MAGNIFICATION OF ORGANIC CHEMICALS BY MOSQUITO FISH IN A MODEL AQUATIC ECOSYSTEM

Compound	Ecological magnification[a]
Aldrin	1,312
Aniline	6
Anisole	22
Benzoic acid	21
Chlorobenzene	650
DDT	16,950
2,6-Diethylaniline	124
Hexachlorobenzene	1,166
Nitrobenzene	29
Pentachlorophenol	296
Phthalic anhydride	0
3,5,6-Trichloro-2-pyridinol	16

[a] Ratio of concentration of pentachlorophenol in mosquito fish to concentration of pentachlorophenol in water.

Source: Adapted from Lu and Metcalf, 1975, Table 1, p. 270. Reprinted by permission of the publisher.

Lu and Metcalf (1975) noted that degradation of hexachlorobenzene by members of the model community resulted in the formation of substantial amounts of pentachlorophenol (Table D.5.27). The presence of pentachlorophenol as a metabolic breakdown product of hexachlorobenzene has a precedent in higher organisms (Section D.6.2.2.5). Thus, the possibility that pentachlorophenol levels in water may result from detoxification of hexachlorobenzene should not be ignored. If hexachlorobenzene serves as a

TABLE D.5.27. DISTRIBUTION OF HEXACHLOROBENZENE AND DEGRADATION PRODUCTS IN A MODEL AQUATIC ECOSYSTEM

Compound	R_f[a]	Hexachlorobenzene equivalent (ng/g)					
		Culture water	Alga (*Oedogonium cardiacum*)	Daphnia (*Daphnia magna*)	Mosquito (*Culex quinquefasciatus*)	Snail (*Physa* sp.)	Fish (*Gambusia affinis*)
Total ^{14}C		26.8	43,700	15,800	36,900	27,600	17,000
Hexachlorobenzene	0.83	9.34	37,100	10,600	24,500	25,000	10,900
Unknown 1	0.72	0.31	1,430	4,940	1,350	1,190	
Pentachlorophenol	0.42	0.25	2,330		1,790		
Unknown 2	0.35		1,090				
Unknown 3	0.26	0.08			152	450	
Unknown 4	0.10		Trace				
Unknown 5	0.05	0.22	655		1,330	331	1,470
Polar	0.0	9.60	1,110	9,360	7,810	674	4,670
Unextractable		6.95					

[a] Retardation factor determined by thin-layer chromatography in a solvent consisting of acetone and benzene in ratio of 50:50 (v/v).

Source: Adapted from Lu and Metcalf, 1975, Table 8, p. 277. Reprinted by permission of the publisher.

source of pentachlorophenol, this mechanism may account for the ubiquitous presence of pentachlorophenol in the environment.

D.5.2.2.4 Detection and Avoidance Reactions — Fish are capable of detecting sodium pentachlorophenate and demonstrate an avoidance reaction to it in a gradient tank. Species differ, however, in the pentachlorophenol level which can be detected. Goodnight (1942) reported that various minnows are able to detect sodium pentachlorophenate at levels greater than 10 mg/liter; below 5 mg/liter, sodium pentachlorophenate was not detected. Tomiyama and Kawabe (1962) reported that "guchi" fish can detect pentachlorophenol at levels as low as 0.2 mg/liter. Coté (1972) reported an avoidance response in juvenile Atlantic salmon when they were exposed to 0.1 mg/liter sodium pentachlorophenate. The ability to avoid high pentachlorophenol concentrations may increase the survival of fish exposed to point sources of pentachlorophenol or sodium pentachlorophenate, but it is of little use against low-level, widespread contamination of waterways. Levels of pentachlorophenol which cause chronic toxicity are below the threshold detection limits of fish.

D.5.2.2.5 Carcinogenicity, Teratogenicity, and Mutagenicity — No carcinogenic or mutagenic effects of pentachlorophenol on aquatic organisms have been demonstrated. Developmental abnormalities in freshwater fish exposed to pentachlorophenol have been described (Section D.5.2.2.2.3).

SECTION D.5

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D.6 BIOLOGICAL ASPECTS IN HUMANS

D.6.1 METABOLISM

D.6.1.1 Uptake and Absorption

D.6.1.1.1 Inhalation — Atmospheric pentachlorophenol can be present as a vapor, a dust, or an aerosol. The small, but substantial, vapor pressure of pentachlorophenol — 0.00011 to 0.12 mm Hg at 20°C to 100°C (Bevenue and Beckman, 1967) — may allow toxic levels of vapor to build up in hot, enclosed areas. The maximum atmospheric concentration recommended by the American Industrial Hygiene Association (1970) is 0.5 mg pentachlorophenol or sodium pentachlorophenate per cubic meter of air. The maximum allowable concentration suggested by Plakhova (1966) of the Soviet Union is 0.1 mg/m^3. Minimum lethal concentrations in air have not been defined. Concentrations in air which do not cause death can cause painful irritation of the nose. This general upper respiratory distress, including violent coughing and sneezing, should prevent individuals from inhaling vapor or dust concentrations which produce adverse systemic effects. However, some tolerance is found in persons who routinely handle these materials. Dust and mist concentrations of >1 mg/m^3 pentachlorophenol or sodium pentachlorophenate cause distress in unacclimated persons (American Industrial Hygiene Association, 1970); concentrations as high as 2.4 mg/m^3 can be tolerated by those conditioned to exposure. Most information on routes of entry of pentachlorophenol into the body deals with dermal exposure and accidental or intentional ingestion. Some entry of pentachlorophenol by the respiratory route was inferred in the following studies.

Gordon (1956) reported nine cases of pentachlorophenol poisoning from 1953 to 1956. Five persons died within 16 to 30 hr following onset of symptoms. Three of the five fatalities occurred from spraying sodium pentachlorophenate on sugar cane or pineapple fields. One death resulted from spraying weeds with a highly concentrated pentachlorophenol–diesel oil mixture, and the fifth fatality occurred following the preparation of a pentachlorophenol formulation. The three fatalities which resulted from spraying sodium pentachlorophenate probably involved some skin contamination, but pathological changes of the lungs were noted at autopsy. Autopsy findings in two cases revealed that the lungs had "the histological appearance of gross congestion and widespread intraalveolar hemorrhage" (Gordon, 1956). Some contamination through the respiratory route was inferred although concentrations of pentachlorophenol in the air were not known. Of the four nonfatal cases reported, two involved pineapple spraying and one occurred at a sawmill, apparently from operation of kilns in which timber soaked in sodium pentachlorophenate solution was dried. The timber was not handled by hand; therefore, skin contamination was unlikely.

Blair (1961) described three cases of sodium pentachlorophenate poisoning in Southern Rhodesia, two of which were fatal. The affected persons were involved in spraying a sodium pentachlorophenate solution on a farm to control snails. One fatality resulted from gross skin contamination with the concentrated solution. The other two were preparing a solution from sodium pentachlorophenate powder under very windy conditions. The powder was blown into their faces, causing smarting of the eyes and irritation of the nose; one of the workers died soon afterwards.

Mason et al. (1965) reported two fatal cases of pentachlorophenol poisoning at a chemical plant. The workers were packaging the powder and "inhaled considerable amounts of dust particles of the chemical." Autopsy findings were not remarkable; in both cases, however, congested, edematous lungs with areas of marked alveolar hemorrhage were found.

Nine cases of fatal pentachlorophenol intoxication occurred in Sarawak, Borneo (Menon, 1958). Although gross skin contamination was common among workers dipping timber in pentachlorophenol preservative solutions, autopsy findings in two of the nine fatalities showed congestion and acute edema of the lungs. The author inferred a respiratory factor in these two fatal intoxications.

Respiratory uptake also apparently was involved in a fatal pentachlorophenol poisoning in Winnipeg, Canada (Bergner, Constantinidis, and Martin, 1965). The individual worked in a factory which made window sashes and wooden frames. All wood was treated with a wood preservative containing 4.1% pentachlorophenol before it was shipped. The worker dipped the wood in the preservative with bare hands, and overt skin contamination occurred. However, it was also stated that "a drying pan with a fan above it was provided, where the wood dried at room temperature, but there was a distinct odour in the room and ventilation appeared to be inadequate. This impression was later confirmed by special ventilation studies."

A clearer case of poisoning by inhalation of pentachlorophenol vapor was reported in Sacramento, California (Anonymous, 1970). A woman, after moving into a new house, noticed that her house plants began to die. In turn, she began to lose weight rapidly and to become weak. Three months later, after losing 9 kg, she was hospitalized. The diagnosis was pentachlorophenol poisoning. A preservative had been applied by the builder to the interior redwood of the home to prevent its turning gray, and a strong odor was present. Ingestion of the compound seems highly unlikely, but some dermal exposure cannot be ruled out. It cannot be inferred that the woman was only exposed to pentachlorophenol vapor. Pentachlorophenol solutions that use light solvents such as mineral spirits do not always contain enough cosolvents (antiblooming agents) to hold the pentachlorophenol in solution in the wood. When this occurs, pentachlorophenol will recrystallize on the surface of the wood (blooming). One must examine the wood carefully for a white to light gray, dustlike surface residue. These small particles can become airborne through brushing, air currents, or household cleaning activities. These crystals also increase the surface area available for vaporization of pentachlorophenol.

A common feature of most of the above cases is an elevated ambient temperature. Bergner, Constantinidis, and Martin (1965) suggested that the increased volatility of pentachlorophenol at higher temperatures results in greater atmospheric quantities of pentachlorophenol available for inhalation.

Pentachlorophenol absorption by the respiratory route was examined critically by Casarett et al. (1969). Two persons were exposed to pentachlorophenol in an enclosed area where the preservative was applied with a brush. Ambient levels of pentachlorophenol in the air and respiratory rates were monitored (Table D.6.1 and Figure D.6.1). No marked change in the pentachlorophenol concentration in the urine occurred until about one day after exposure; peak values occurred one or two days after exposure.

TABLE D.6.1. INHALATION OF PENTACHLOROPHENOL BY TWO HUMAN SUBJECTS AND RECOVERY IN URINE

Pentachlorophenol value	Subject A	Subject B
Mean air concentration, $\mu g/m^3$	230	432
Calculated exposure dose, μg	91	147
Recovered in urine, μg	80[a]	112[b]
Recovered in urine, %	88	76

[a] Recovered 160 hr after exposure.
[b] Recovered 120 hr after exposure.

Source: Adapted from Casarett et al., 1969, Table III, p. 363. Reprinted by permission of the publisher.

Not unexpectedly, pentachlorophenol has been found in the air of wood treating plants. Wyllie et al. (1975) sampled air at 11 sites at five different times in a plant treating 2.5 million board feet of lumber annually. Average pentachlorophenol levels ranged from 263 to 1888 ng/m^3 (0.26 to 1.8 $\mu g/m^3$). The highest level reported was 15 $\mu g/m^3$ in a sample from the pressure treating room. The air samples were collected for an average of 6 hr. Pentachlorophenol levels in the dip tank area ranged from 12 to 800 ng/m^3 and in storage areas from 9 to 900 ng/m^3. Pentachlorophenol in serum of workers was on the order of 1 to 2 mg/liter; urinary levels ranged from 0.08 to 0.3 mg/liter. The highest level in serum was 3.9 mg/liter. Levels in a control were 0.04 to 0.06 mg/liter in serum and 0.004 mg/liter in urine.

A survey of workers in a wood preservation operation indicated a substantial pentachlorophenol content in the blood and urine as compared

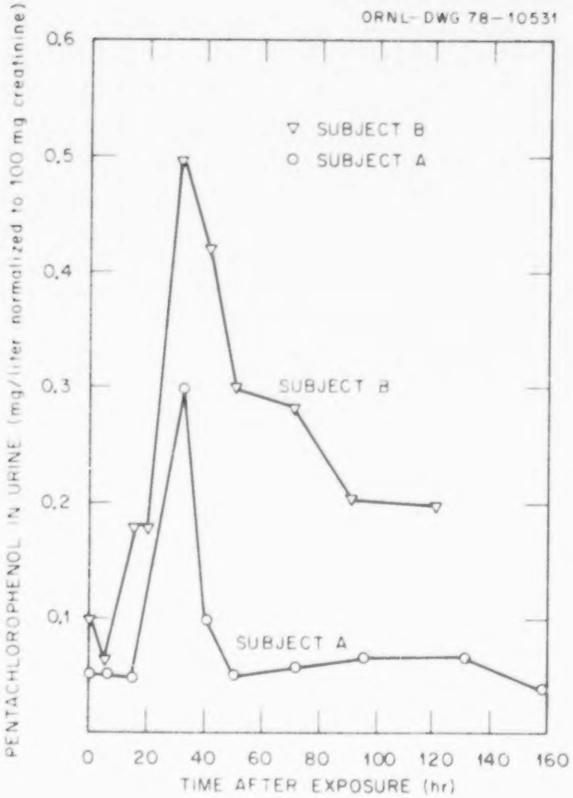

Figure D.6.1. Pentachlorophenol in urine of two subjects following respiratory exposure. Source: Adapted from Casarett et al., 1969, Figure 3, p. 363. Reprinted by permission of the publisher.

with nonoccupationally exposed people (Figure D.6.2). Casarett et al. (1969) stated, "The workers in this study unquestionably sustain some dermal exposure, although protective gloves and barrier creams are used. Data from air samples obtained in the plants were not given because the method of sampling was not properly characterized. Nevertheless, they demonstrated qualitatively that significant concentrations of pentachlorophenol were present in the plant atmosphere. Under the conditions of widespread dissemination in the yard, and the prevalent temperature and wind, at least part of the exposure of the workers must be considered to occur via the respiratory tract."

Plakhova (1966) reported that the average lethal concentration of sodium pentachlorophenate was 240 mg/m^3 for mice and 341 mg/m^3 for guinea pigs. Exposure of animals (type not given) to sodium pentachlorophenate at a concentration of 23 mg/m^3 for 4 hr daily for four months resulted in a decrease in weight, erythrocytes, and hemoglobin and in an increase in leucocytes and eosinophils.

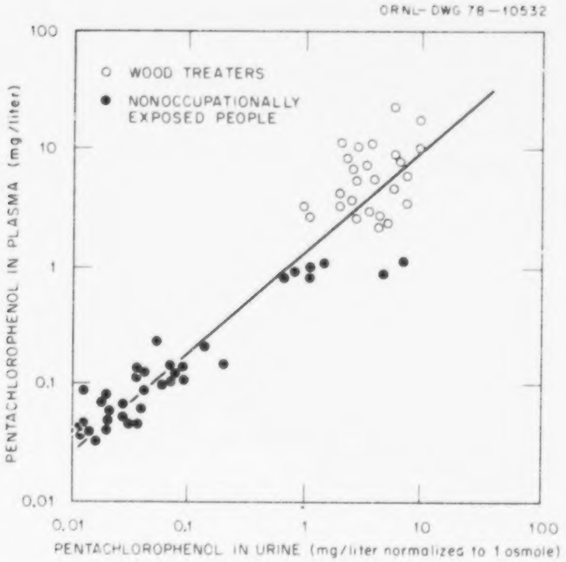

Figure D.6.2. Pentachlorophenol in paired blood and urine samples of wood treaters and nonoccupationally exposed people. Source: Adapted from Casarett et al., 1969, Figure 5, p. 364. Reprinted by permission of the publisher.

Hoben, Ching, and Casarett (1976*a*, 1976*b*, 1976*c*) and Hoben et al. (1976) reported an experimental method for evaluating the toxicity of sodium pentachlorophenate inhaled by rats. These authors claimed an LD_{50} for an inhaled sodium pentachlorophenate aerosol of 11.7 mg/kg body weight. This conclusion, however, is difficult to substantiate based on the methods and results presented. The paper describing the experiment (Hoben, Ching, and Casarett, 1976*b*) does not state the aerosol concentration nor the vehicle used to dissolve the sodium pentachlorophenate. The authors stated that the aerosol mixture was continuously sampled throughout the experiments and that desired doses of sodium pentachlorophenate were achieved by varying the exposure time. Unfortunately, neither the actual aerosol concentrations nor exposure times were reported. Only one of these four documents reports the actual aerosol concentration (i.e., when the exposure chamber was calibrated) (Hoben, Ching, and Casarett, 1976*a*): "To test reproducibility of the aerosol output a PCP solution containing 10 g PCP, 6 ml glycerol and 1.7 g NaOH in 100 ml water was atomized four times for periods of 20 minutes. The aerosol was collected at a flow rate of 0.5 l/min. The results lay between 761 and 802 mg PCP with a standard error of 1.7%." If one calculates the dose of sodium pentachlorophenate delivered to rats under these conditions for the reported 28- to 44-min exposures (Hoben, Ching, and Casarett, 1976*b*), the values ranged from 795 mg/kg body weight to 1249 mg/kg body weight. The large discrepancy between these calculated values and the reported LD_{50} of 11.7 mg/kg body weight suggests that the actual inhalation experiments were conducted under much different conditions. It is not possible to resolve this question based on the data provided and attempts to contact the authors were unsuccessful. Thus, the reported LD_{50} value must be considered questionable.

Except for the suggestion by Bergner, Constantinidis, and Martin (1969) that increased ambient temperatures result in increased volatility of pentachlorophenol and sodium pentachlorophenate, causing increased concentrations in the atmosphere, no investigations defined the factors responsible for the uptake of pentachlorophenol or sodium pentachlorophenate from the atmosphere. Specific quantitative data concerning the effect of the chemical and physical forms of these substances on absorption by the respiratory tract are not available.

D.6.1.1.2 Ingestion — Pentachlorophenol is repellent to laboratory animals. The food intake by rats decreased when their diet contained pentachlorophenol, and cats refused salmon treated with pentachlorophenol (Deichmann et al., 1942). The level in the rat food was approximately 300 to 600 mg/kg. At 125 to 250 mg/kg, cats refused to eat, but levels equivalent to 30 to 60 mg/kg were apparently consumed.

Experiments with animals confirm that absorption from the gastroenteric tract is common after ingestion of pentachlorophenol. Kehoe, Deichmann-Gruebler, and Kitzmiller (1939) administered an 11% solution of pentachlorophenol in olive oil to rabbits. They stated that since the rectal temperatures of these animals rose slowly, the absorption of pentachlorophenol from the gastrointestinal tract was slow. Conversely, investigations by Deichmann et al. (1942) indicated that the absorption of sodium pentachlorophenate (18 mg/kg) from the stomach of the rabbit was almost immediate, reaching a peak of 2.4 mg per 100 g of blood in 7 hr (Figure D.6.3). Absorption began almost immediately following a dose of sodium pentachlorophenate equivalent to 18 mg/kg or more. The blood level reached a peak after about 7 hr when a dose of 37 mg/kg was administered. The findings of Machle, Deichmann, and Thomas (1943) confirm the rapid appearance of sodium pentachlorophenate in blood and urine of rabbits following oral administration (Figure D.6.4).

Translocation from the abdominal cavity to the digestive tract has been demonstrated in mice (Jakobson and Yllner, 1971). Carbon-14—labeled pentachlorophenol was injected intraperitoneally, and the tissue distribution of the compound was followed by autoradiography. Within 4 hr after injection, pentachlorophenol was detected in the fundus wall of the stomach and in the stomach and intestinal contents.

Braun et al. (1977) showed that the half-life for absorption of $[^{14}C]$pentachlorophenol after ingestion by rats was 1.3 ± 0.4 hr; the peak plasma concentration occurred 4 hr after ingestion. A dose of 0.1 mg/kg resulted in a peak plasma level of 0.248 mg/liter. The calculated 99% of steady-state value was 0.491 mg/liter, which would be reached after 8.4 days of ingestion of 0.1 mg/kg per day. Braun and Sauerhoff (1976) administered a single oral dose of 10 mg $[^{14}C]$pentachlorophenol per kilogram in corn oil to three male and three female rhesus monkeys.

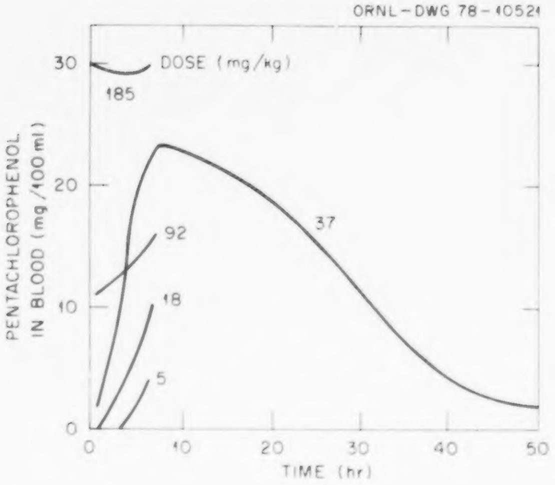

Figure D.6.3. Pentachlorophenol in blood of rabbits after a single oral dose of sodium pentachlorophenate. Source: Adapted from Deichmann et al., 1942, Figure 1, p. 110. Reprinted by permission of the publisher.

The half-lives for absorption were 3.6 hr in the male and 1.8 hr in the female.

No studies addressed the question of how the physical form of pentachlorophenol affects its absorption across the intestinal tract. Some suggestive evidence can be obtained, however, relating to the relative toxicities of sodium pentachlorophenate and pentachlorophenol. The toxicity of pentachlorophenol is approximately twice as great as that of sodium pentachlorophenate administered by the oral or cutaneous routes. According to Kehoe, Deichmann-Gruebler, and Kitzmiller (1939), the smallest oral dose of pentachlorophenol in olive oil resulting in the deaths of rabbits was 110 mg/kg; the smallest lethal dose of sodium pentachlorophenate in aqueous solution was 250 to 300 mg/kg. Similar findings were reported by Deichmann et al. (1942) and by Dow Chemical Company (1969*a*, 1969*b*). This difference in toxicity was not apparent in guinea pigs (Flickinger, 1971). The toxicities of pentachlorophenol and sodium pentachlorophenate are similar when the compounds are administered intravenously and intraperitoneally; thus, the difference in toxicity of the two forms administered via the oral or cutaneous route may result from differential absorption by the intestinal tract and the skin.

Reports of human intoxication with pentachlorophenol via the oral route are rare. Three of nine fatal cases reported by Menon (1958) appeared to involve ingestion. The individuals involved were engaged in a timber dipping operation with pentachlorophenol preservative solution. Although overt, gross skin contamination was common, autopsy reports indicated inflamed gastric mucosa; thus, a contribution to intoxication by the oral route was inferred. A report from Australia (Gordon, 1956)

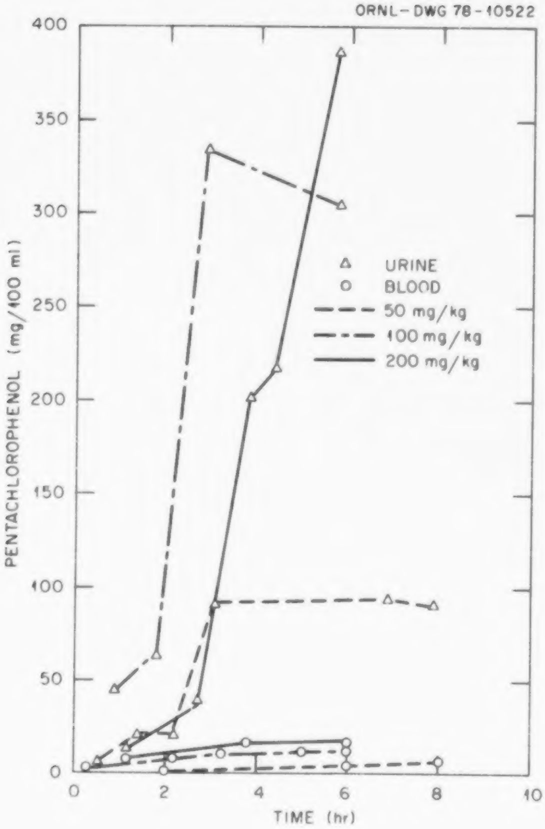

Figure D.6.4. Pentachlorophenol in blood and urine of rabbits exposed to a single oral dose. Source: Adapted from Machle, Deichmann, and Thomas, 1943, Figures 13, p. 193. Reprinted by permission of the publisher.

also suggested intoxication by the oral route. The individual was mixing a 1% solution of sodium pentachlorophenate. Some of the solution was siphoned into another container, and apparently some was swallowed. Death ensued about 9 hr after onset of symptoms.

A clear-cut case of pentachlorophenol poisoning by ingestion was reported by Blair (1961). The case involved an African who died in July 1960 as a result of consuming food contaminated accidentally with sodium pentachlorophenate. He had collected maize meal from a grain storage bin in the dark and had prepared maize meal porridge for himself and a friend. Early the next morning the African called for help and was found to be sweating profusely. He did not vomit or have convulsions and died about 1 hr later. Later inspection revealed that a small tin of "insecticide" used against rodents and insects was present in the grain bin. It was found, in fact, to contain technical grade sodium pentachlorophenate. The maize meal porridge was later analyzed and was found to contain 216 mg of sodium pentachlorophenate per gram of porridge.

Ingestion of pentachlorophenol is probably rare among humans. Only in fairly exceptional circumstances, such as suicidal intent or extraordinary insensitivity, is someone likely to ingest a compound of such noxious character. If taken internally, vomiting would probably result before much of the compound could be absorbed (Flickinger, 1971). However, possible ingestion of extremely small amounts of pentachlorophenol in the diet cannot be ignored. In areas where sodium pentachlorophenate or pentachlorophenol is widely applied, contamination of food crops is possible. The significance of these small amounts of pentachlorophenol which may be consumed by human populations is discussed in Section D.6.2.2.4.2. Levels in food and estimated dietary intake of pentachlorophenol are discussed in Sections D.6.2.2.5 and D.8.2.

D.6.1.1.3 Cutaneous Absorption — Most of the reported cases of pentachlorophenol intoxication in humans involved direct absorption of pentachlorophenol or its sodium salt through the skin. According to the American Industrial Hygiene Association (1970), "Solutions of either as dilute as about 1% may cause irritation if contacts are prolonged or repeated frequently. However, solutions as dilute as 0.1% are not likely to cause adverse local effects. Absorption through the skin is reported if the contact is prolonged beyond five or ten minutes or for repeated contacts for shorter time periods. If contacts are continued over an extended period of time (months to years), an 'acne-like' dermatitis is possible."

The first incident of serious pentachlorophenol intoxication was reported by Truhaut, Boussemart, and L'Épée (1952). The individuals affected were involved in a timber preservation operation in southwestern France. The timber was dipped in 3% solutions of sodium pentachlorophenate and tetrachlorophenate without protection for the workers. The men immersed their hands and forearms in the solution, and their vests and overalls were contaminated. Pentachlorophenol was found in amounts ranging from 3 to 10 mg/liter in the urine of 16 men who had been working for two months. The toxic effects were severe enough to cause death in two cases.

Gordon (1956) reported nine cases of pentachlorophenol poisoning in Australia, five of which were fatal. The workers were spraying pineapple fields with an aqueous solution of sodium pentachlorophenate. They wore shorts, often without a shirt, and frequently wore no shoes. In this particular method, the spray was directed towards the ground, which perhaps reduced inhalation, but this certainly increased contamination of the lower limbs.

Skin absorption of pentachlorophenol was implicated in two fatalities reported by Bergner, Constantinidis, and Martin (1965). The men were employed by a firm which manufactured window sashes. These sashes, prior to shipment, were dipped in a preservative solution consisting of pentachlorophenol, a petroleum solvent, and unnamed inert ingredients.

In both cases, the workers were involved in dipping operations and did not wear gloves.

A definite case of pentachlorophenol absorption through the skin was reported by Bevenue, Haley, and Klemmer (1967). A man cleaned a paint brush with a solution which, it was subsequently determined, contained pentachlorophenol. Analysis of the solution used to clean the brush indicated a 0.4% concentration of pentachlorophenol and a trace amount of tetrachlorophenol, which suggested that the paint thinner or brush cleaner solvent had been added to a can that contained a residual amount of pentachlorophenol. Skin irritation persisted despite a thorough washing. Two days following exposure a 24-hr urine specimen was analyzed. Subsequent urine samples (each was the first void in the morning) were also analyzed. The data indicate that pentachlorophenol was absorbed through the skin and that elimination of the compound from the body was gradual (Table D.6.2).

Chapman and Robson (1965) reported a case of serious intoxication in England. The patient, a girl 3.5 years of age, developed fever, intermittent delirium, and rigors during the night. After she was admitted to a hospital, urine analysis indicated a pentachlorophenol content of 6 mg per 100 ml of urine. Examination of the house showed

TABLE D.6.2. RESIDUES OF PENTACHLOROPHENOL IN URINE OF INDIVIDUAL AFTER CUTANEOUS ABSORPTION

Date sampled	Pentachlorophenol in urine[a] (μg/liter)	Residue as ratio of initial analysis[b] (%)
1-17-67	236	
1-19-67	80	34
1-20-67	90	38
1-30-67	48	20
2-6-67	64	27
2-17-67	23	10
3-7-67	17	7

[a] Data not corrected for percent recoveries.

[b] Recovery values of fortified samples were 89% to 92% with detection limits of 3 μg/liter.

Source: Adapted from Bevenue, Haley, and Klemmer, Table 1, p. 295. Reprinted by permission of the publisher.

that the cold-water storage tank in the roof was contaminated with an insecticide mixture that had been used to spray the roof timbers before the family moved in, some 13 days previously. The mixture contained pentachlorophenol, β-naphthol, and dieldrin in a tractor oil—like solvent. Scum was evident on the bath water. Drinking water was obtained from the public water main; therefore, except for brushing her teeth, the patient was unlikely to have drunk much contaminated water. Absorption through the skin was the most probable route of uptake. This is a case of multiple intoxication. Dieldrin, as well as pentachlorophenol, is absorbed through the skin, and the neurologic signs reported are more consistent with dieldrin toxicosis than with pentachlorophenol toxicosis. The high pentachlorophenol content of the urine also indicates significant pentachlorophenol exposure.

From April to August 1967, 20 newborns had an unusual disease characterized by profuse generalized diaphoresis, fever, tachycardia, tachypnea, hepatomegaly, and acidosis (Armstrong et al., 1969). Nine of the infants were affected seriously and two died. Intense investigation of the hospital premise resulted eventually in the identification of a laundry product which contained sodium pentachlorophenate. An antimicrobial laundry neutralizer containing pentachlorophenol had been used in the final rinse of hospital linens. The ingredients listed on the labels of two 45-kg drums of powder were as follows: sodium pentachlorophenate, 22.9%; 3,4,4'-trichlorocarbanilide, 4.0%; sodium salts of other chlorophenols, 3.2%; and inert ingredients, 69.9%. High concentrations of this product were found in diapers and hospital linens. High pentachlorophenol levels were detected in the serum and urine of infants, nurses, and expectant mothers exposed to the linens (Table D.6.3). According to Armstrong et al. (1969), "Sodium pentachlorophenate is readily absorbed through the skin, and, since it is a water-soluble substance, its absorption would be enhanced by urine or moisture on the skin." Armstrong et al. (1969) suggested that the increased toxicity of these compounds to infants was a result of their relatively immature renal function, which might have caused pentachlorophenol to accumulate. In addition, pentachlorophenol excreted in urine may have been reabsorbed from wet diapers. For example, a 10-min exposure of hands to 0.4% pentachlorophenol resulted in urine concentrations of 236 mg/liter (Bevenue, Haley, and Klemmer, 1967).

According to Dow Chemical Company (1969*a*, 1969*b*), solid pentachlorophenol is not absorbed through the skin in acutely toxic amounts, but cutaneous contact with solid sodium pentachlorophenate can result in systemic intoxication. One report appears to contradict these statements. Nomura (1953) reported one fatal case and two nonfatal cases of systemic intoxication by pentachlorophenol. The workers were involved in centrifuging, drying, and packaging pentachlorophenol following its manufacture. Extensive skin contact was believed to be responsible for the poisonings.

Animal studies confirm that pentachlorophenol and sodium pentachlorophenate are easily absorbed via the cutaneous route. Table D.6.4 summarizes the toxicity of pentachlorophenol and sodium pentachlorophenate in

TABLE D.6.3. LEVELS OF PENTACHLOROPHENOL IN SERUM AND URINE FROM HEALTHY PERSONS AT HOSPITAL WHERE A PENTACHLOROPHENOL POISONING INCIDENT OCCURRED

Persons sampled	Time relationship to discontinuation of antimicrobial laundry neutralizer	Pentachlorophenol level (mg/liter)			
		Serum		Urine	
		Mean	Range	Mean	Range
6 infants	-2 days	18.7	11.3-25.6	0.34	0.02-0.70
9 infants	+2 weeks	9.2	3.3-17.5	0.10	0.01-0.66
1 infant	+8 weeks	2.0		0.06	
5 nurses	-2 days	5.4	1.7-12.6		
5 nurses	+8 weeks	0.9	0.5-1.9	0.08	0.04-0.14
2 expectant mothers	+8 weeks	1.9	1.8-2.0	0.37	0.28-0.46

Source: Adapted from Armstrong, R. W., E. R. Eichner, D. E. Klein, W. F. Barthel, J. V. Bennett, V. Jonsson, H. Bruce, and L. E. Loveless, 1969, Pentachlorophenol Poisoning in a Nursery for Newborn Infants: II. Epidemiologic and Toxicologic Studies, J. Pediatr. 75:317-325. Reprinted by permission of the publisher.

various solvents to experimental animals. The toxicity of pentachlorophenol, as defined by the minimum lethal dose, varies depending on the solvent used. It appears to be most toxic in petroleum solvents. It is impossible to compare directly the toxicity of pentachlorophenol and sodium pentachlorophenate via the cutaneous route because the two compounds have different solubilities in any given solvent. A general comparison, however, indicates that pentachlorophenol in organic solvents is considerably more toxic to experimental animals than sodium pentachlorophenate in aqueous solution. No investigations addressed the question of why the organic solvent affected the cutaneous absorption of pentachlorophenol. Kehoe, Deichmann-Gruebler, and Kitzmiller (1939) investigated the possibility that the specific gravities of the solvents or their ability to dissolve pentachlorophenol may affect cutaneous toxicity, but no correlations were found. Diechmann et al. (1942) noted that soap and water were more effective in removing pentachlorophenol or sodium pentachlorophenate from the skin than was alcohol. Investigations of the mechanism by which these compounds pass through the skin and specific quantitative data are badly needed.

D.6.1.2 Transport and Distribution

D.6.1.2.1 In Blood — Whether pentachlorophenol is taken up through the skin, via the respiratory tract, or through ingestion, it is distributed in and transported by the blood. Substantial data indicate that

TABLE D.6.4. MINIMUM LETHAL DOSES OF PENTACHLOROPHENOL AND SODIUM PENTACHLOROPHENATE APPLIED CUTANEOUSLY TO EXPERIMENTAL ANIMALS

Animal	Composition of solution applied	Minimum lethal dose (mg/kg)	Time required for death (hr)	Source
		Pentachlorophenol		
Albino rabbit	5% in olive oil	>180[a]		Deichmann et al., 1942
	5% in 95% ethanol	>150[a]		Deichmann et al., 1942
	10% in 95% ethanol	>1111[a]		Deichmann et al., 1942
	10% in 99% ethanol	>309[a]		Deichmann et al., 1942
	10% in corn oil	>326[a]		Deichmann et al., 1942
	5% in No. 1 fuel oil	60–70	1.5–4	Deichmann et al., 1942
	5% in furnace oil	90–100	1.5–3	Deichmann et al., 1942
	5% in No. 3 fuel oil	130–170	6	Deichmann et al., 1942
	5% in Dione oil	110–120	5–6.5	Deichmann et al., 1942
	5% in pale paraffin oil	>150[a]		Deichmann et al., 1942
	1.8% in pine oil	40–50	9–22	Deichmann et al., 1942
Rabbit	Solid	200[b]		Dow Chemical Company, 1969
	In organic solvent	100–200[c]		Dow Chemical Company, 1969
	5% in fuel oil	60–170		Flickinger, 1971
	11% in olive oil	350		Flickinger, 1971
	20% in prophylene glycol	50–200[c]		Flickinger, 1971
		Sodium pentachlorophenate		
Rabbit	10% aqueous	250	3–8	Deichmann et al., 1942
	2% aqueous	257	2.5	Keohe, Deichmann-Gruebler, and Kitzmiller, 1939
	10% aqueous	450–600	1–20	McGavack et al., 1941
	20% aqueous	100–300[d]		Dow Chemical Company, 1969
	10% aqueous	200		Flickinger, 1971
Guinea pig	2% aqueous	266	1	Keohe, Deichmann-Gruebler, and Kitzmiller, 1939

[a] Minimum lethal dose was not achieved.
[b] Absorption of pentachlorophenol was not detected.
[c] At lower value no animals died; at upper value all animals died.
[d] Value is an LD_{50}.

pentachlorophenol is present in the blood following uptake by any of these routes. Autopsies of victims poisoned by pentachlorophenol have shown invariably the presence of pentachlorophenol in the blood. Pentachlorophenol is excreted primarily in the urine; analyses of urine and blood following administration of pentachlorophenol to experimental animals show a rough parallel between urine and blood concentrations. It has been suggested that urinary concentrations be used as a tool for diagnosing pentachlorophenol exposure in humans.

Machle, Deichmann, and Thomas (1943) studied the relationship between blood and urinary concentrations of pentachlorophenol in rabbits (Figure D.6.4). Single oral doses of sodium pentachlorophenate were administered to rabbits. Eight hours after administration of 50, 100, and 200 mg of sodium pentachlorophenate, the blood levels were 4, 10, and 13 mg per 100 ml, respectively, and the urinary concentrations were 92, 344, and 372 mg per 100 ml respectively. These data demonstrate a sharp rise in urinary pentachlorophenol in response to a slight increase in the level of pentachlorophenol in the blood. This correlation between urinary and blood concentrations has been shown before and is the reason for the suggestion that urinary determinations may offer a sensitive means of estimating the extent of human absorption of pentachlorophenol.

Accumulation of pentachlorophenol in the blood following repeated small doses has been shown (Deichmann et al., 1942). Daily feeding of pentachlorophenol (3 mg/kg body weight) to rabbits for 90 days caused accumulation of the compound in concentrations up to 0.6 mg per 100 ml. A threshold level was seen, however. Machle, Deichmann, and Thomas (1943) demonstrated that rabbits given 90 daily doses of sodium pentachlorophenate at a level of 1 mg pentachlorophenol per kilogram of body weight resulted in levels of 1.5 to 3.0 mg/liter in blood.

Pentachlorophenol is apparently not bound to the cellular constituents of blood (Bevenue et al., 1968; Larsen et al., 1972). It is eliminated fairly rapidly by the urinary route in both experimental animals and in humans. However, a study of human excretion rates indicates the possibility of a two-component urinary excretion (Figure D.6.5). This two-component excretion curve led to the suggestion by Casarett et al. (1969) that a binding of pentachlorophenol to plasma proteins may account for the second, slower excretion rate. This suggestion is particularly important because it indicates that some pentachlorophenol would be available for redistribution to tissues following an initial rapid excretion.

Casarett et al. (1969) examined the urine and plasma of nonoccupationally exposed persons and of workers in wood treating plants in Hawaii. Pentachlorophenol concentrations in plasma and in urine were uniformly higher among exposed persons (Figure D.6.2). However, a low concentration of pentachlorophenol was discovered in the urine and plasma of nonoccupationally exposed persons (Section D.6.2.2.4.2). This study suggests a ratio of pentachlorophenol in blood to pentachlorophenol in urine of between 1.5 and 2.5 (Figure D.6.2).

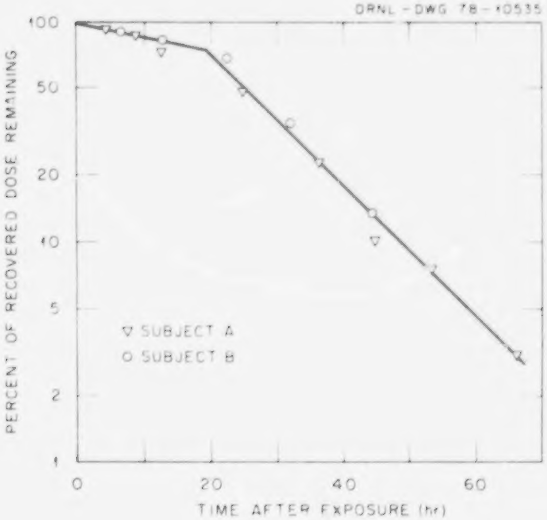

Figure D.6.5. Pentachlorophenol remaining in two human subjects after inhalation exposure. Source: Adapted from Casarett et al., 1969, Figure 4, p. 364. Reprinted by permission of the publisher.

Higher pentachlorophenol concentrations in urine than in blood are frequently reported for acute intoxications. Animal studies with a single dose also show this pattern. Two possible explanations for this reversal are (1) an effect of pentachlorophenol on the kidney or (2) sufficient amounts of pentachlorophenol are carried into urine by plasma proteins from the blood to cause this effect. Casarett et al. (1969) stated that the data in Figure D.6.6 indicate the binding of pentachlorophenol with elements of the blood: "Blood and urine PCP concentrations were linearly related up to about 0.1 ppm, above which the plasma levels reached a plateau approaching 100 ppm with increasing levels of PCP in the urine. These data suggest the possibility of binding to plasma proteins....With an apparent upper limit of plasma concentration and some limitation of urinary excretion rates, it is quite probable that some of the PCP would be available for redistribution to tissues."

Braun et al. (1977) administered single oral doses of [^{14}C]pentachlorophenol in corn oil to rats at rates of 10 and 100 mg/kg. Peak plasma concentrations of 30 to 40 mg/liter were reached in 4 to 6 hr after the 10 mg/kg exposure. They demonstrated that 95% of the plasma pentachlorophenol was bound to protein. In monkeys given a single dose of 10 mg/kg, peak plasma levels of 10 to 30 mg/liter were attained 12 to 24 hr after administration. From the pharmacokinetic model, it was calculated that 90% of the plasma steady state would be reached after ten daily doses, and the approximate plasma concentration would be 45 mg/liter.

D.6.1.2.2 Organ Distribution — Data for tissue distribution following human uptake of pentachlorophenol are derived mainly from autopsies

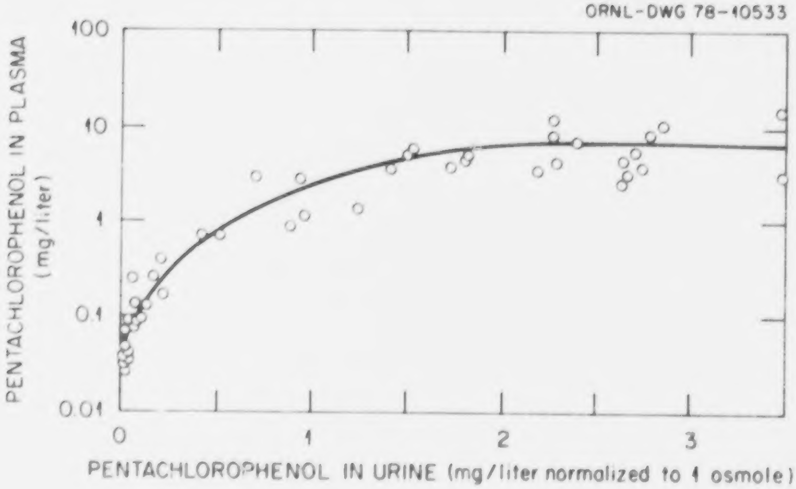

Figure D.6.6. Relationship between pentachlorophenol concentrations in plasma and urine. Source: Adapted from Casarett et al., 1969, Figure 6, p. 365. Reprinted by permission of the publisher.

of fatal cases of pentachlorophenol poisoning. Tables D.6.5 through D.6.8 summarize autopsy findings in seven cases of fatal pentachlorophenol intoxication. It is, of course, difficult to base conclusions on data of this type. However, the presence of pentachlorophenol in the liver, kidneys, and — in some cases — the stomach is a common finding. The presence of pentachlorophenol in other tissues may depend on the route of exposure.

From these data, levels of pentachlorophenol associated with acute lethal toxicosis can be estimated. Levels in the blood, liver, and kidney are most meaningful. Levels in urine can vary depending on how much urine was in the bladder at the time of ingestion. Residues associated with death are 50 to 176 mg/kg in blood, 62 to 225 mg/kg in the liver, and 28 to 123 mg/kg in the kidney. Haley (1977) reported an attempted suicide by a 71-year-old male whose peak level of pentachlorophenol in blood was 155 mg/kg. He was saved by an aggressive therapy that included gastric lavage, diuretics, maintenance of electrolyte balance, and intravenous fluids to promote renal excretion.

A study by Armstrong et al. (1969) is interesting because it includes the only analysis of fatty tissue from a human acutely exposed to pentachlorophenol. The case involved an infant exposed to lethal concentrations of sodium pentachlorophenate in diapers and hospital linens (Section D.6.1.1.3). The data indicate a 25% greater concentration of pentachlorophenol in fat than in other tissues (Table D.6.6). Significant concentrations of pentachlorophenol have been found in the adipose tissue of nonoccupationally exposed persons (Shafik, 1973). This aspect of pentachlorophenol accumulation has not been investigated thoroughly.

TABLE D.6.5. AUTOPSY ANALYSES OF TWO HUMANS FATALLY POISONED BY PENTACHLOROPHENOL

Subject	Specimen	Pentachlorophenol (mg/100 g tissue)	
		Detected	Corrected average[a]
A	Blood	10.8, 11.1	15.6
	Liver	8.7, 9.1	13.4
	Kidney	8.0, 9.2	12.3
	Brain	2.0, 2.9	3.5
	Urine	36, 37	52
B	Blood	8.3, 7.5	11.3
	Liver	6.7, 6.4	9.4
	Brain	1.2, 0.74	1.4
Control	Liver fortified with 9.28 mg/100 g of pentachlorophenol	6.3 (69% recovery)	

[a] Correction for recovery value = pentachlorophenol detected x 1.43.

Source: Adapted from Mason et al., 1965, Table IV, p. 145. Reprinted with permission from the Journal of Forensic Sciences, April 1965, published by Callaghan & Company, 3201 Old Glenview Road, Wilmette, Illinois 60091.

TABLE D.6.6. CONTENT OF PENTACHLOROPHENOL IN TISSUES OF A FATALLY POISONED INFANT (mg/100 g)

Tissue	Pentachlorophenol
Kidney	2.8
Adrenal	2.7
Heart and blood vessel	2.1
Fat	3.4
Connective tissue	2.7

Source: Adapted from Armstrong, R. W., E. R. Eichner, D. E. Klein, W. F. Barthel, J. V. Bennett, V. Jonsson, H. Bruce, and L. E. Loveless, 1969, Pentachlorophenol Poisoning in a Nursery for Newborn Infants: II. Epidemiologic and Toxicologic Studies, J. Pediatr. 75:317-325. Reprinted by permission of the publisher.

TABLE D.6.7. AUTOPSY REPORT ON 16-YEAR-OLD PATIENT FATALLY POISONED WITH PENTACHLOROPHENOL

Macroscopic appearance

Trachea: Slight congestion and edema were present in lower third of trachea extending into both main bronchi.

Lungs: Slight congestion and edema were present in both lower lobes, but otherwise nothing significant was seen.

Liver: On the cut surface there were some ill-defined, pale brownish yellow areas which were scattered throughout the liver tissue; these were irregular in shape and stood out in contrast to the darker normal liver tissue. The cut surface had a somewhat cloudy and glossy appearance.

Microscopic appearance

Heart: Some peculiar degeneration of intravascular leucocytes and some fragmentation of occasional muscle fibers were found.

Lung: Some passive congestion, patchy collapse, and slight alveolar hemorrhage were seen.

Analyst report

Pentachlorophenol content: Blood, 5 mg/100 ml; urine, 7 mg/100 ml; lung, 14.5 mg/100 g; kidney, 9.5 mg/100 g; liver, 6.5 mg/100 g; brain, 2.0 mg/100 g.

Source: Adapted from Gordon, 1956, Table III, p. 487. Reprinted by permission of the publisher.

TABLE D.6.8. AUTOPSY FINDINGS FROM THREE PERSONS FATALLY POISONED WITH PENTACHLOROPHENOL
(mg/100 ml or mg/100 g)

Specimen	Pentachlorophenol content		
	Subject A	Subject B	Subject C
Liver	6.2	5.9	5.9
Lung	7.6		
Kidney	8.4	4.1	6.3
Blood (from lung)	9.7		
Blood (from liver)	4.6	5.3	
Urine (from bladder)		2.8	
Stomach			7.8

Source: Compiled from Blair, 1961.

Specific data on the tissue distribution of pentachlorophenol administered by various routes to experimental animals have been reported in several studies. Two studies investigated the quantity of pentachlorophenol in fat of experimental animals. Larsen et al. (1972) examined the tissue distribution of [^{14}C]pentachlorophenol in rats following oral administration. A low level of pentachlorophenol was found in fat relative to the concentration in other tissues. Measurable levels of pentachlorophenol were found in the fat of female Golden Syrian hamsters up to 120 hr following oral doses of pentachlorophenol (Hinkle, 1973). The amount found following the 20 mg/kg exposure was not specified.

Braun and Sauerhoff (1976) recovered 11.7% and 11.2% of a 10 mg/kg dose in the tissues of two female monkeys 360 hr after administration. The largest amounts were found in the liver (9.72%) and in the small and large intestines (7.66%). Other tissues, including brain, fat, muscle, bone, and remaining soft tissues, contained only 2% to 3.5% of the dose. In rats, nine days after a single 10 mg/kg dose, 0.44% remained in the body, with 82% of the residue located in the liver and kidney (Braun et al., 1977). In a study using one male and one female rat necropsied at 4, 24, 48, 72, and 120 hr after dosing, the highest levels in tissues were in liver and kidney and the lowest levels were in brain, spleen, and fat. Except for the liver in female rats and the liver and kidney in male rats, pentachlorophenol levels were higher in plasma than in organs.

Pentachlorophenol was present in almost every tissue of rabbits 24 hr following a lethal oral dose of a 13.5% solution of pentachlorophenol (Flickinger, 1971). Deichmann et al. (1942) analyzed the tissues of six rabbits which died accidentally two to seven days following the beginning of an experiment in which 3 mg pentachlorophenol per kilogram body weight was fed. Analyses indicated concentrations of 0.4 to 0.8 mg pentachlorophenol per 100 ml of blood, 0.15 to 0.4 mg per 100 g in liver tissue, and 0 to 0.3 mg per 100 g in kidney tissue. Following repeated cutaneous applications of pentachlorophenol to rabbits, Deichmann et al. (1942) found 0.5 to 26 mg/kg in the kidneys, lungs, and liver (Table D.6.9). Analyses of rabbit tissues 24 hr following oral administration of sodium pentachlorophenate showed that the largest amounts of pentachlorophenol (expressed as percent of amount ingested) were located in the stomach and intestines, muscles, skin, and bones (Table D.6.10). Smaller amounts were found in the kidney and in the liver and gallbladder combined. However, when pentachlorophenol levels were expressed as the concentration in rabbit tissues (i.e., micrograms of pentachlorophenol per gram of tissue), kidney and liver-gallbladder samples contained the highest pentachlorophenol concentrations among the tissues analyzed.

Examination of the "percentage of total dose per tissue" data for mice in Table D.6.11, taken from Jakobson and Yllner (1971), reveals that the highest levels of pentachlorophenol, following intraperitoneal injection, were in the stomach, intestines, and liver. Other organs showed, at most, 2% of the total dose. These investigators noted that "concentrations" of pentachlorophenol in the tissues (micrograms of pentachlorophenol per gram organ weight) were highest for the liver and gallbladder. Very high levels in the gallbladder (three to four times

TABLE D.6.9. CONCENTRATION OF PENTACHLOROPHENOL IN TISSUES OF RABBITS AFTER CUTANEOUS APPLICATION OF 10 ml OF 1% AQUEOUS SODIUM PENTACHLOROPHENATE FOR 100 CONSECUTIVE DAYS, EXCEPT SUNDAYS
(mg/100 g tissue)

Specimen	Pentachlorophenol content	
	Rabbits washed 0.5 hr after application	Rabbits washed 1 hr after application
Urine	4.9	6.6
Blood	0.23	0.45
Kidney	0.14	0.26
Lung	0.14	0.09
Liver	0	0.05
Brain	0	0
Muscle	0.06	0

Source: Adapted from Deichmann et al., 1942, Table 4, p. 108. Reprinted by permission of the publisher.

TABLE D.6.10. DISTRIBUTION OF PENTACHLOROPHENOL IN TISSUES OF TWO RABBITS FOLLOWING ORAL DOSES OF SODIUM PENTACHLOROPHENATE
(% of amount ingested)

Specimen	Pentachlorophenol recovered	
	Rabbit A[a]	Rabbit B[b]
Urine and feces	70.6	
Urine		70.3
Feces		0.3
Stomach and intestine (wall and contents)	6.8	3.9
Muscle	6.4	3.6
Bone	3.1	1.3
Skin	2.2	3.9
Blood	1.9	5.7
Liver and gallbladder	0.74	2.0
Kidney	0.27	0.38
Heart, lung, and testes	0.17	0.32
Central nervous system	0.096	0.088
Total	92.2	91.1

[a] Received 94 mg pentachlorophenol.
[b] Received 97 mg pentachlorophenol.

Source: Adapted from Deichmann et al., 1942, Tables 6 and 7, p. 113. Reprinted by permission of the publisher.

TABLE D.6.11. EXCRETION AND DISTRIBUTION OF [^{14}C]PENTACHLOROPHENOL IN MICE AFTER INTRAPERITONEAL INJECTION[a]

Specimen	Pentachlorophenol content									
	No. 1[b]		No. 2[b]		No. 3[b]		No. 4[c]		No. 5[d]	
	% of dose	µg/g	% of dose	µg/g	% of dose	µg/g	% of dose	µg/g	% of dose	µg/g
Urine										
0–24 hr	45		45		54		60		59	
24–48 hr	30		16		12		11		15	
48–72 hr	5.0		6.1		4.2		5.8		4.3	
72–96 hr	3.4		4.6		2.3		3.1		1.4	
96–168 hr							1.8		0.9	
Feces										
0–24 hr	0.1		4.2				6.5			
24–96 hr	7.7		0.8							
0–96 hr					3.8					
24–168 hr							3.2			
0–168 hr									12	
Liver	2.2	9	2.5	10	3.1	26	1.2	10	0.4	3
Kidney	0.09	2	0.5	8	0.04	1	0.04	1	0.01	0.3
Lung	0						0.02	1	0	
Heart	0.03	2			0		0.01	1	0.01	1
Brain	0.01	0.1					0.02	0.5	0	
Stomach (with contents)			2.1	21	0.5	10				
Gallbladder (with contents)			0.08	60	0.06	90				
Intestine (with contents)			4.8	8	2.2	8				
Residue	5.0		0.6		2.0		1.4		0.1	
Total	98.1		87.6		84.8		93.9		92.4	

[a] Doses administered: No. 1, 14.8 mg/kg; No. 2, 18.2 mg/kg; No. 3, 37.2 mg/kg; No. 4, 35.2 mg/kg; No. 5, 36.8 mg/kg.

[b] Killed after 96 hr.

[c] Killed after 7 days.

[d] Killed after 30 days; urine and feces analyzed for first week.

Source: Adapted from Jakobson and Yllner, 1971, Table 1, p. 518. Reprinted by permission of the publisher.

the concentration found in any other tissue) indicate liver metabolism. In view of the high pentachlorophenol levels found in the gallbladder of experimental animals, it is suggested that the gallbladder might serve as an indicator organ in establishing pentachlorophenol exposure.

The data of Larsen et al. (1972) were expressed in a slightly different fashion (Figure D.6.7). The content of pentachlorophenol in the tissues of rats receiving an oral dose was expressed as the mean percentage of dose per gram of tissue. Again, high levels of pentachlorophenol were found in the liver, stomach, and intestines, and also in the kidneys.

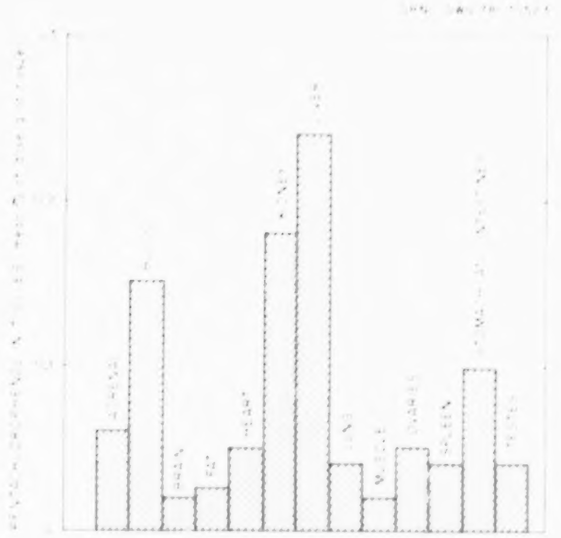

Figure D.6.7. Tissue distribution of [^{14}C]pentachlorophenol and/or its labeled metabolites 40 hr after oral administration to rats. Source: Adapted from Larsen et al., 1972, Figure 2, p. 2005. Reprinted by permission of the publisher.

High levels of pentachlorophenol in the liver, stomach, intestines, kidneys, and gallbladder can be explained in several ways. The compound may be present in the kidneys as a result of their function in urinary excretion. Its presence in the liver may arise from pentachlorophenol detoxification. From whole-body autoradiograms of mice subcutaneously injected with [^{14}C]pentachlorophenol, Jakobson and Yllner (1971) found:

> Four hours after the injection the highest specific activities were found in the fundus wall of the stomach and in the stomach and intestinal contents. During 4 hours there was a passage throughout the gastrointestinal tract....A high specific activity was also recorded for the liver....The blood and kidneys displayed moderate activity, the lungs and brain little, if any.

> After 20 hours the blackening corresponding to the wall of the stomach fundus was considerably weaker but the contents of the stomach, and especially the intestines, still caused marked blackening. A considerable amount of activity was collected in the liver and moderate amounts were still recorded in the blood and kidneys.
>
> After 100 hours the distribution was largely the same as after 20 hours. The strongest blackening was registered for the intestinal contents and the liver. In the kidneys it was moderate....
>
> A number of organic compounds, most of them basic, are secreted by gastric juice....The high specific activity in the wall of the stomach fundus and the stomach contents evident in the autoradiograms shows that PCP is also secreted in this way....The high specific activity of the gall bladder and its contents, however, shows that biliary secretion is also important in the metabolism of PCP. The excretion of PCP and its metabolites thus occurs via the kidneys and by the processes of gastric and biliary secretion; the greater part being passed in the urine.

The findings of Deichmann et al. (1942) are consistent with this hypothesis even though the liver and gallbladder were analyzed together. Larsen et al. (1972) demonstrated activity in the liver but could not analyze gallbladder tissue because rats lack this organ. The transport and distribution scheme proposed by Jakobson and Yllner (1971) (Figure D.6.8) thus appears consistent with present information. A modified scheme (Figure D.6.9) includes the possibility that pentachlorophenol is bound to serum proteins in the blood and the evidence for gastric and biliary secretion (Casarett et al., 1969). If entry into the blood from the skin and lungs were included, this diagram would seem to account for

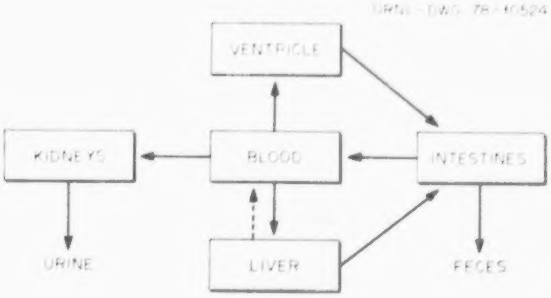

Figure D.6.8. Turnover of pentachlorophenol and/or its metabolites in the mouse. Source: Adapted from Jakobson and Yllner, 1971, Figure 4, p. 523. Reprinted by permission of the publisher.

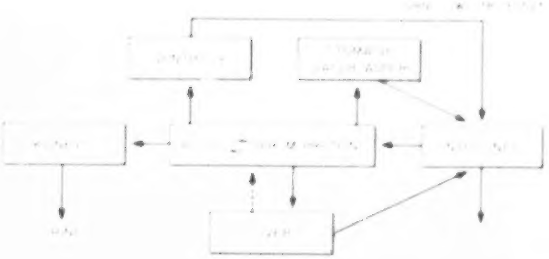

Figure D.6.9. Proposed turnover of pentachlorophenol and/or its metabolites in mammals.

all transport mechanisms in the mammalian system with the possible exception of placental transfer. The following statements summarize the transport and distribution of pentachlorophenol in humans and experimental animals:

1. Pentachlorophenol is distributed in some quantity throughout the body tissues.
2. Highest tissue levels are found in the liver, kidney, and blood.
3. There is evidence of enterohepatic circulation which may prolong the elimination of a small amount of absorbed pentachlorophenol.
4. There is no evidence to suggest a significant long-term accumulation in body fat of mammals such as that which occurs with some chlorinated hydrocarbon chemicals.

D.6.1.2.3 Placental Transfer — Transfer of pentachlorophenol across the placenta has not been adequately studied, and complete data have not been reported. Investigations by Hinkle (1973) indicated that pentachlorophenol can cross the placental barrier and enter the fetus. Oral doses of pentachlorophenol ranging from 1.25 to 20 mg/kg body weight were administered daily to Golden Syrian hamsters. Samples of maternal blood and fat and entire fetuses were analyzed. According to Hinkle (1973), "There was close correlation between the concentrations of PCP recovered from the maternal blood and the fetuses. The highest measured concentrations in the blood, fat and fetuses occurred within 3 hr following the last administered oral dose. Concentrations in fat persisted in measureable amounts up to 120 hr following the last oral dosing and exceeded the blood and fetal concentrations at that time." Unfortunately, quantitative data were not given.

Larsen et al. (1975) found a negligible placental transfer of pentachlorophenol in rats. Radiolabeled pentachlorophenol was administered orally in a solution of olive oil at a dose of 68 mg/kg body weight, which is approximately 75% of the reported LD_{50} (Figure D.6.10). The authors believed that the amounts found in the placentas probably

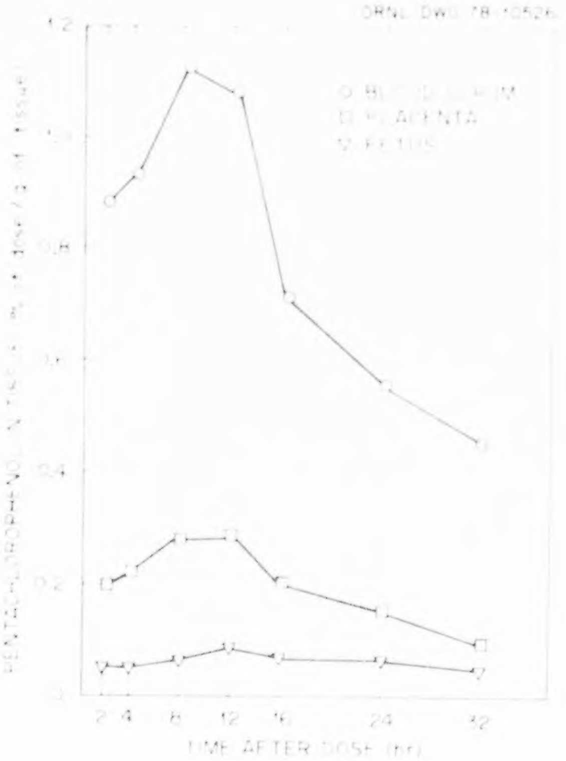

Figure D.6.10. [^{14}C]Pentachlorophenol and/or its metabolites in tissues after an oral dose to pregnant rats on day 15 of gestation. Source: Adapted from Larsen et al., 1975, Figure 1, p. 125. Reprinted by courtesy of Marcel Dekker, Inc.

resulted largely from the blood content. Figure D.6.10 shows that the pentachlorophenol levels of the fetuses remained very low throughout the experiment. It is difficult to assess the validity of the conflicting reports by Hinkle (1973) and Larsen et al. (1975). It is not clear in the Hinkle (1973) study whether or not placental tissue was included in the analysis under the category of entire fetuses. If it were included, contamination of fetal tissue by placental blood could explain the fetal levels of pentachlorophenol.

D.6.1.3 Biotransformation

Table D.6.12 summarizes biotransformation data and pentachlorophenol compounds found in the urine of experimental animals and humans. Pentachlorophenol is principally eliminated in the urine except for very high oral doses, in which case significant amounts appear in the feces. Amounts of unchanged pentachlorophenol found in urine range from 100% for the rhesus monkey down to 40% in the mouse. Other compounds include tetrachlorohydroquinone and pentachlorophenol or tetrachlorohydroquinone

TABLE D.6.12. PENTACHLOROPHENOL BIOTRANSFORMATION IN MAMMALS

Species	Sex	Dose (mg/kg)	Route	Peak blood level (ppm)	Time to peak level (hr)	Plasma half-life (hr)	Excretion in urine and feces	Metabolites found	Source
Man		0.1	Oral	0.248	4	30.2	Peak at 42 hr; half-life for pentachlorophenol 33 hr; half-life for pentachlorophenol glucuronide 12.7 hr	74% in urine as pentachlorophenol, 12% in urine as pentachlorophenol glucuronide, 4% in feces as pentachlorophenol and pentachlorophenol glucuronide	Braun, Blau, and Chenoweth, 1978
Monkey	Male	10	Oral	10-30	12-24	72	Urine half-life 41 hr	In urine as unchanged pentachlorophenol, no metabolites	Braun and Sauerhoff, 1976
	Female	10		10-30	12-24	84	Urine half-life 92 hr; 358 hr after single dose, 70% in urine, 18% in feces, and 11% in tissues		Braun and Sauerhoff, 1976
Rat		25	Intraperi-toneal	NR	NR	NR	74% in urine at 24 hr	41% as unchanged pentachlorophenol, 5% as tetrachlorohydroquinone, 10% as tetrachlorohydroquinone conjugate, and 14% as pentachlorophenol conjugate in body	Ahlborg, Lindgren, and Mercier, 1974
	Male	10	Oral	45	4-6	17 (1) 40 (2)	80% in urine, 19% in feces		Braun et al., 1977
	Female	10	Oral	45	4-6	13 (1) 30 (2)	78% in urine, 19% in feces		Braun et al., 1977

(continued)

[illegible]

Species	Sex	Dose (mg/kg)	Route	Peak blood level (ppm)	Time to peak level (hr)	[illegible]	[illegible]	[illegible]	[illegible]
	Male	100	Oral			[illegible]	[illegible]	[illegible]	[illegible]
	Female	100	Oral			[illegible]	[illegible]	[illegible]	[illegible]
Mouse		25		NR	NR	NR	[illegible]	[illegible]	[illegible]
		15-47	Intraperitoneal or subcutaneous	NR	NR	NR	[illegible]	[illegible]	[illegible]

[a]NR = Not reported.

conjugates. Some studies have found the conjugates to include glucuronides; others have not identified the conjugates.

The earliest investigation of pentachlorophenol metabolism was reported by Deichmann et al. (1942). The rate of destruction of pentachlorophenol was monitored in rats and rabbits. A colorimetric method of analysis based on the reaction of pentachlorophenol with fuming nitric acid following steam distillation of acidified specimens was used. Following oral administration of sodium pentachlorophenate to rabbits, 50% to 75% of the ingested pentachlorophenol was excreted in the urine and feces over a 24-hr period (Figure D.6.11). Analysis of pentachlorophenol in the carcasses revealed that 10% of the ingested pentachlorophenol was unaccounted for and presumably was metabolized. Rats given an intraperitoneal injection of pentachlorophenol excreted approximately 12.5% over a 24-hr period; analysis of carcasses showed that 40% of the injected pentachlorophenol had been metabolized (Table D.6.13). In other experiments, Deichmann et al. (1942) found that detoxification through conjugation to sulfuric or glucuronic acid did not occur; no other metabolites were detected.

Jakobson and Yllner (1971) have shown that pentachlorophenol can be detoxified in mice by conjugation and metabolism. Labeled compounds in

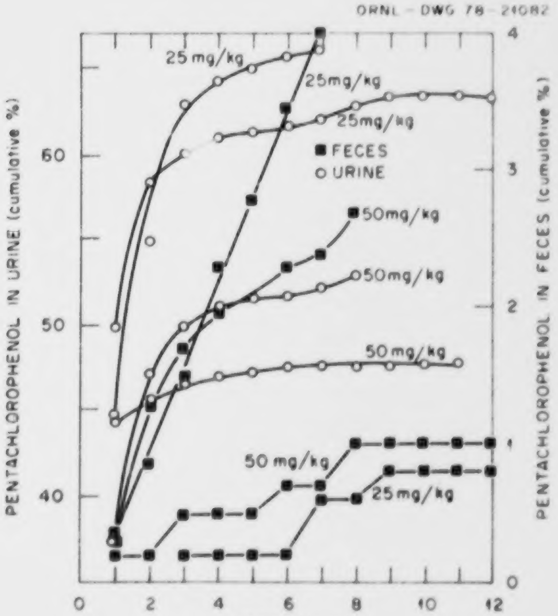

Figure D.6.11. Excretion of pentachlorophenol by rabbits after oral administration of sodium pentachlorophenate. Source: Compiled from Deichmann et al., 1942, Table 8, p. 114.

TABLE D.6.13. RECOVERY OF PENTACHLOROPHENOL IN THE CARCASSES AND EXCRETA OF RATS FOLLOWING A SINGLE INTRAPERITONEAL DOSE OF 2.4 mg PENTACHLOROPHENOL PER ANIMAL

Time animal killed after treatment (hr)	Pentachlorophenol (% of amount injected)		
	In excreta	In carcass	Metabolized
0	0	100	0
3	7.5	83	10
12	6.6	68	25
24	13	47	41
48	17	23	61
72	13	18	68
96	8.3	15	77
120	9.5	13	78
144	12	13	75

Source: Adapted from Deichmann et al., 1942, Table 9, p. 115. Reprinted by permission of the publisher.

urine, collected 0 to 24 hr after intraperitoneal injection of [^{14}C]pentachlorophenol were identified using an isotope dilution technique before and after paper chromatographic separation. These investigators found that about 60% of the injected isotope activity was excreted in the urine in 24 hr. About 30% of the injected pentachlorophenol appeared in the urine as unchanged pentachlorophenol (Table D.6.14). Acid hydrolysis of the urine and subsequent analysis yielded an additional 8% of the total dose, which apparently resulted from pentachlorophenol conjugates; the nature of these conjugates was not determined. Approximately 21% of the injected ^{14}C activity consisted of ^{14}C-labeled tetrachlorohydroquinone; this compound was possibly conjugated in the urine Because the sample was insufficient, only one determination of tetrachlorohydroquinone in the urine was made prior to hydrolysis, and the extent of tetrachlorohydroquinone conjugation could not be assessed. The presence of pentachlorophenol and tetrachlorohydroquinone in the urine was confirmed by paper chromatography utilizing two different solvent systems. From the recovery values of ^{14}C activity, it was apparent that pentachlorophenol and its conjugates and tetrachlorohydroquinone and its conjugates accounted for >97% of the urinary activity excreted. The findings of Jakobson and Yllner (1971) are consistent with the data for rats reported by Deichmann et al. (1942). In summary, degradation of pentachlorophenol 24 hr following administration to rats and mice was 40% and 30%

TABLE D.6.14. CARBON-14 ACTIVITY IN URINE COLLECTED 0 TO 24 hr AFTER INTRAPERITONEAL INJECTION OF [^{14}C]PENTACHLOROPHENOL IN MICE

Dose (mg/kg)	Pentachlorophenol		Pentachlorophenol conjugates		Tetrachlorohydroquinone and conjugates		Total urinary ^{14}C activity (%)
	% of dose	% of urinary ^{14}C	% of dose	% of urinary ^{14}C	% of dose	% of urinary ^{14}C	
36.3[a]	29	50	9	16	26	48	106
17.0[a]	30	54	7	12	15	33	99
7.9[b]	26[c]	54[c]			21	44	98[d]

[a] Mean for four mice.

[b] Mean for three mice.

[c] Pentachlorophenol and pentachlorophenol conjugates determined together.

[d] Obtained from determination of pentachlorophenol + conjugates = 54%.

Source: Adapted from Jakobson and Yllner, 1971, Table 2, p. 520. Reprinted by permission of the publisher.

respectively. In the rabbit, 8% of the administered dose was degraded in 24 hr. Intraperitoneal injection was the route of uptake for both the rat and the mouse; the different rates of degradation noted in the rabbit following oral administration could be due either to species differences or to the route of entry of the compound.

Similarly, Ahlberg, Lindgren, and Mercier (1974) gave mice and rats intraperitoneal doses of [^{14}C]pentachlorophenol and found that approximately 70% of the injected activity was excreted in the urine in 24 hr. Analysis of the components with a gas chromatograph–mass spectrometer technique showed that 40% of the excreted activity was unchanged pentachlorophenol (Table D.6.15). A second major component, tetrachlorohydroquinone, accounted for 24% of the excreted activity in the mice and 5% in the rats. Following hydrolysis by boiling with concentrated hydrochloric acid, a third major component consisted of a mixture of pentachlorophenol and tetrachlorohydroquinone conjugates. Following hydrolysis, the total pentachlorophenol and tetrachlorohydroquinone contents of the urine accounted for 100% of the excreted ^{14}C activity. The distribution of pentachlorophenol and its conjugates and tetrachlorohydroquinone and its conjugates in urine was approximately 50% to 50%. Thus, the rat produces much more tetrachlorohydroquinone conjugate than the mouse.

If urinary excretion is used as a measure of occupational exposure in humans, pentachlorophenol, its metabolite tetrachlorohydroquinone, and their conjugates should all be assessed. Pentachlorophenol glucuronide has also been detected as a pentachlorophenol metabolite in human urine (Braun, Blau, and Chenoweth, 1978). The metabolism and pharmacokinetics of pentachlorophenol were determined in four male volunteers who ingested 0.1 mg pentachlorophenol per kilogram. Approximately 74% of the dose was eliminated in the urine as pentachlorophenol and 12% was eliminated in the urine as pentachlorophenol glucuronide. Additionally, 4% was eliminated in feces as pentachlorophenol and pentachlorophenol glucuronide. Urine from persons exposed to pentachlorophenol was analyzed by Ahlborg, Lindgren, and Mercier (1974). Pentachlorophenol and tetrachlorohydroquinone were quantified and their identities confirmed. Earlier data on the pentachlorophenol content of human urine may have been underestimated by a factor of 2.

Braun et al. (1977) administered [^{14}C]pentachlorophenol to rats and determined the urinary metabolites. Seventy-five percent of the pentachlorophenol in the urine was unchanged, 16% appeared as tetrachlorohydroquinone, and 9% was present as pentachlorophenol glucuronide. In the plasma most of the pentachlorophenol was unchanged; a small amount of pentachlorophenol glucuronide was present. Tetrachlorohydroquinone was not detected in blood plasma. The rhesus monkey eliminated pentachlorophenol unchanged in the urine with no metabolites reported following a single oral dose (10 mg) of pentachlorophenol (Braun and Sauerhoff, 1976).

Tashiro et al. (1970) identified a strongly acidic pentachlorophenol metabolite in urine of rabbits administered sodium pentachlorophenate

TABLE D.6.15. CARBON-14 ACTIVITY IN URINE OF RATS AND MICE RECEIVING 10 TO 25 mg/kg PENTACHLOROPHENOL IN A SINGLE INTRAPERITONEAL DOSE

Animal	Treatment of urine	^{14}C activity in urine (%)		
		As pentachlorophenol	As tetrachlorohydroquinone	As pentachlorophenol and tetrachlorohydroquinone conjugates
Rat	None	43	5	52
	HCl at 100°C	57	43	
Mouse	None	41	24	35
	HCl at 100°C	54	46	

Source: Adapted from Ahlborg, Lindgren, and Mercier, 1974, Table 2, p. 277. Reprinted by permission of the publisher.

orally; small amounts of chloroanil, a compound closely related to tetrachlorohydroquinone, were also found. The acidic metabolite was characterized as pentachlorophenyl β-glucuronide, but it decomposes at high temperatures to pentachlorophenol; the conditions for mass spectrometry provide sufficiently high temperatures for this transformation. Treatment of the metabolite with dilute hydrochloric acid yielded pentachlorophenol, glucuronic acid, and glucuronolactone; it also was hydrolyzed by the action of β-glucosidase. This substance was also a major metabolite in the urine of rats dosed subcutaneously with sodium pentachlorophenate. Chloranil and pentachlorophenyl β-glucuronide were incorporated into a proposed metabolic pathway for pentachlorophenol in mammals (Figure D.6.12). This is the only study which specifically reported the conjugation of pentachlorophenol to glucuronic acid in experimental animals. A metabolic pathway similar to that proposed by Tashiro et al. (1970) was outlined by Jakobson and Yllner (1971) (Figure D.6.13). In some ways, all of the data reported appear to be consistent with each of these pathways. In Figure D.6.14, this information is combined into a single integrated metabolic pathway for pentachlorophenol in mammals. Species differences must be kept in mind.

Substantial amounts of pentachlorophenol are found in the liver following different routes of administration. Based on the known role of the liver in detoxification of foreign substances, it is apparent that metabolic breakdown may take place. No studies were found in which the bile duct was cannulated and the pentachlorophenol metabolism in the liver was characterized. However, the work of Jakobson and Yllner (1971) points out both the mobility of pentachlorophenol in body tissues and in the biliary secretion. They injected [^{14}C]pentachlorophenol into mice and found high activity in the stomach fundus, stomach contents, and gallbladder. Pentachlorophenol may also be secreted in the gastric juice (Jakobson and Yllner, 1971). Braun et al. (1977) reported that most of the pentachlorophenol remaining in the rat body was found in the liver eight days after a single 10 mg/kg dose. Braun and Sauerhoff (1976)

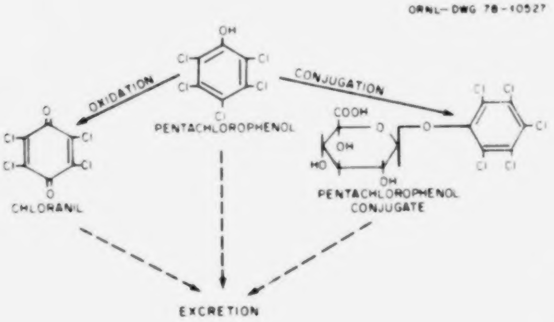

Figure D.6.12. Proposed metabolic pathway of pentachlorophenol in mammals. Source: Adapted from Tashiro et al., 1970, Figure 5, p. 129. Reprinted by permission of the publisher.

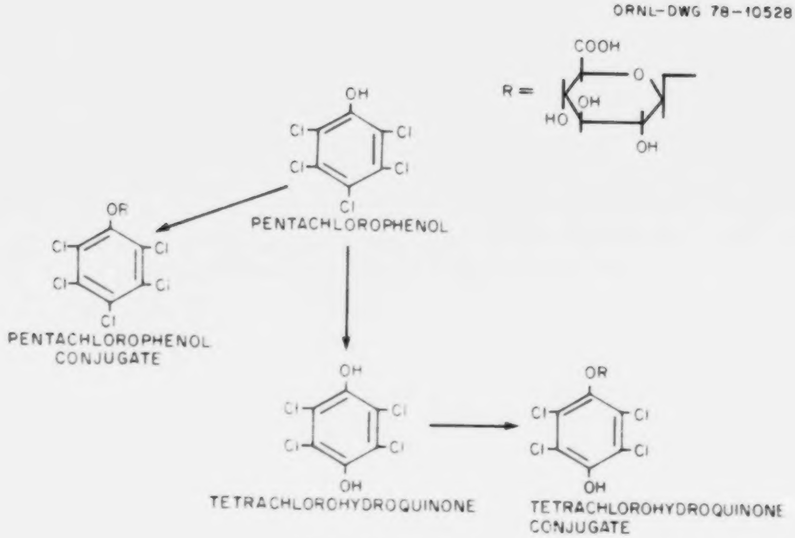
ORNL-DWG 78-10528
R =
COOH
O
OH
HO
OH
OH
Cl
PENTACHLOROPHENOL
OR
PENTACHLOROPHENOL CONJUGATE
TETRACHLOROHYDROQUINONE
TETRACHLOROHYDROQUINONE CONJUGATE

Figure D.6.13. Proposed metabolic pathway of pentachlorophenol in mice. Source: Adapted from Jakobson and Yllner, 1971, Figure 2, p. 522. Reprinted by permission of the publisher.

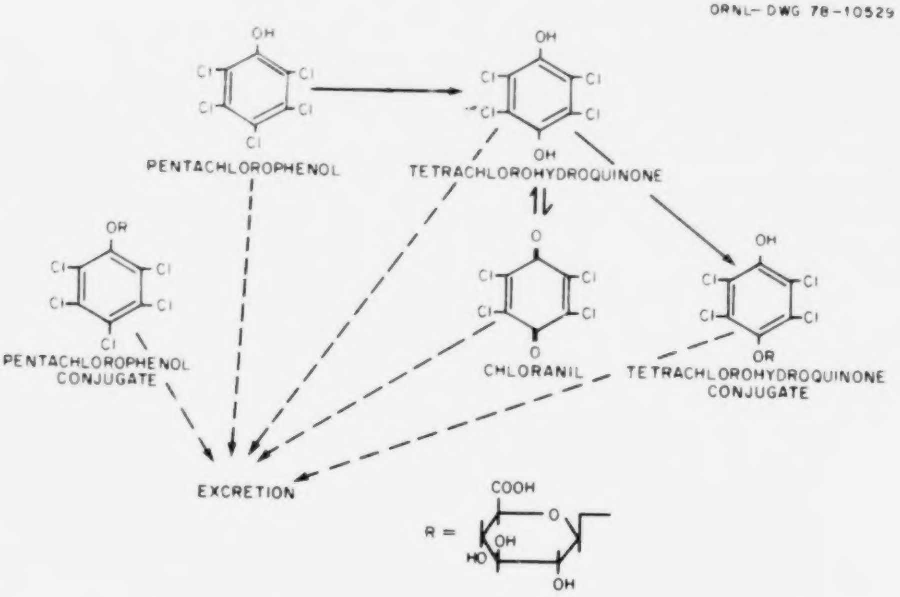
ORNL-DWG 78-10529
OH
Cl
PENTACHLOROPHENOL
TETRACHLOROHYDROQUINONE
OR
PENTACHLOROPHENOL CONJUGATE
O
CHLORANIL
TETRACHLOROHYDROQUINONE CONJUGATE
EXCRETION
R =
COOH
HO

Figure D.6.14. Proposed integrated metabolic pathway of pentachlorophenol in mammals.

demonstrated that the rhesus monkey continues to eliminate some pentachlorophenol in the feces after the initial bolus would have been eliminated; this is further evidence for biliary excretion and enterohepatic circulation. Enterohepatic circulation would contribute to a prolonged half-life of trace-level residues.

D.6.1.4 Elimination

The primary mode of pentachlorophenol excretion appears to be through the urine. Although initial elimination may be very rapid, it has been shown that following exposure to pentachlorophenol a return to background levels may take as much as a month, as in the case of an individual in Hawaii who was exposed while cleaning a paint brush in a solution contaminated with pentachlorophenol (Bevenue, Haley, and Klemmer, 1967) (Table D.6.2). In evaluating the data, these authors stated, "The pentachlorophenol residue in the urine of individuals not knowingly occupationally exposed (201 samplings) had a mean value of 23 pbb. It is noted... from the data...that about one month was required for the pentachlorophenol concentration in the affected individual's urine to decrease to the average amount observed in random samplings of the local male population not occupationally exposed."

Two volunteers were exposed to pentachlorophenol by inhalation (Casarett et al., 1969). Urinary concentrations of pentachlorophenol (normalized to 100 mg of creatinine) were monitored and plotted as the midpoint of the interval during which the urine was collected. The rate of excretion during the first 10 to 24 hr was less than that beyond 24 hr. The data are expressed differently in Figure D.6.5; data from both subjects were sufficiently similar to allow a single line to be drawn through the plotted points. The early rate of excretion indicates an excretion half-life between 40 and 50 hr, but the rate of excretion after 24 hr can be represented by a half-life of approximately 10 hr.

Braun, Blau, and Chenoweth (1978) found the pentachlorophenol half-life in plasma to be 30.2 ± 4.0 hr. The half-lives for elimination of pentachlorophenol and pentachlorophenol glucuronide from urine were 33.1 ± 5.4 hr and 12.7 ± 5.4 hr respectively. The dynamics of elimination in humans were described as a one-compartment, open-system model with first-order absorption, enterohepatic circulation, and first-order elimination.

Bevenue et al. (1967) conducted an extensive survey as part of a community pesticide program in Hawaii. Data on levels of pentachlorophenol in urine of occupationally and nonoccupationally exposed persons were collected. In Hawaii, pentachlorophenol is used extensively for the treatment of wood products to control termites and has been used as a preemergence herbicide in pineapple and sugar cane fields. It is perhaps not surprising, therefore, to find a substantial level of pentachlorophenol in the urine and blood of nonoccupationally exposed Hawaiians (Section D.6.2.4.2). Sequential urinary levels in occupationally exposed persons were examined to identify exposure probabilities among workers and individual variation in excretion patterns. Pairings of samples (one sample taken immediately following exposure) from the sequential urinary level determinations were used to calculate a mean daily rate of decrement in urinary pentachlorophenol (Figure D.6.15). The log of the rate of decrease of urinary pentachlorophenol was plotted against time over which the decrement occurred. Bevenue et al. (1967) stated, "Although a smooth curve has been drawn, two single straight lines can also be drawn because the data can as readily be represented as a combination of two exponential functions. This suggests the possibility of two different excretion rates."

Apparently, a partially independent relationship exists between the amount of pentachlorophenol in the initial sample and the rate of decrease in the urine (Figure D.6.16). If one assumes that the rate of excretion is related to the total body dose or burden, the amount and rate of pentachlorophenol excreted apparently increases as the body content increases. Furthermore, it appears that the rate of excretion is higher when the body burden is sufficiently high to result in an excretion level greater than 3 to 5 mg/liter in the "initial" sample, again implying the possibility of more than one excretion rate. Bevenue et al. (1967) emphasized that more data are necessary to support the suspicion of a "threshold" above which there is a higher excretion rate and below which there is a slower rate. Caution must be observed in interpreting these data because as the authors stated, "If one could assume that the time between samples was the interval following a single exposure, the conclusion that there are two rates of excretion might be justified. However, there is no way to eliminate the possibility that intermittent exposures had occurred in most of the individuals represented...." Such exposures could explain at least part of the apparent change of rate and might even be the entire explanation.

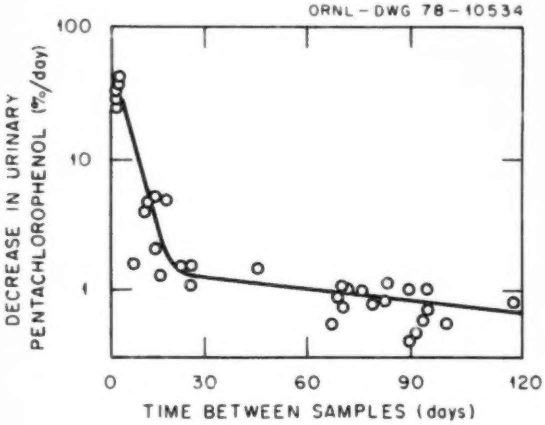

Figure D.6.15. Change in urinary pentachlorophenol with time for occupationally exposed people. Source: Adapted from Bevenue et al., 1967, Figure 2, p. 328. Reprinted by permission of the publisher.

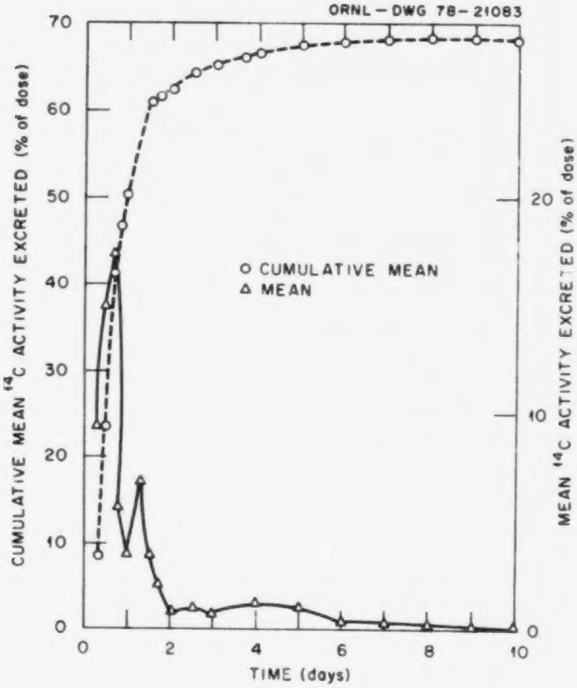

Figure D.6.16. Decrease in urinary pentachlorophenol with time for occupationally exposed people. Source: Adapted from Bevenue et al., 1967, Figure 3, p. 328. Reprinted by permission of the publisher.

Casarett et al. (1969) also conducted an epidemiological study of the blood and urine concentrations of occupationally and nonoccupationally exposed persons in Hawaii. Although it is difficult to draw conclusions from this type of investigation because the total body dose or body burden was unknown, some interesting inferences were drawn. Figure D.6.2 suggests that the ratio of pentachlorophenol in blood to that in urine is between 1.5 and 2.5, but considerable variations appear in individual measurements. Although this ratio is similar to that found in some acute intoxications (Blair, 1961), other reports of acute intoxications gave higher concentrations in the urine than in the blood (Gordon, 1956; Bergner, Constantinidis, and Martin, 1965).

This blood to urine ratio is in marked contrast to the findings in experimental animals. When single doses of pentachlorophenol are administered to experimental animals, urinary levels of pentachlorophenol are invariably higher than blood levels (Machle, Deichmann, and Thomas, 1943). Casarett et al. (1969) also reported that in preliminary animal studies with single doses, higher concentrations of pentachlorophenol are found in urine than in blood. This suggests that if a species difference does not exist, a kinetic difference exists between single and chronic exposures.

Larsen et al. (1972) suggested a two-component urinary excretion of pentachlorophenol in rats following oral administration (Figure D.6.17). In fact, the curve was resolved into two components by fitting the data into an exponential equation (terms not explained):

$$BB(t) = B_1e^{-B_2t} + B_3e^{-B_4t}\ .$$

The first component had a half-life of 10 hr, and the second component had a half-life of 102 days. This study was criticized by Braun et al. (1977). They stated, "These values were obtained from a sigma-minus plot by subtracting the cumulative amount excreted in the urine from the total dose. This approach may cause gross errors in estimating elimination rates unless the total recovery approaches 100% of the dose....Since the total recovery of the dose was not reported in the communication by Larsen and co-workers cited above, the accuracy of the reported 102-day half-life for elimination is questionable."

Braun et al. (1977) described the pharmacokinetics of pentachlorophenol in rats given single oral doses of [^{14}C]pentachlorophenol. Table D.6.16 summarizes the results. A two-compartment open-system model was necessary to describe the data for males given 10 or 100 mg/kg and for females given 10 mg/kg. The results showed a rapid elimination of pentachlorophenol in the urine. Only 0.4% of the dose remained in the body after nine days. This is in contrast to the data of Larsen et al. (1972), who reported 30% of the dose remaining in the body after ten days. The Larsen study did not report total recovery and may have ignored fecal

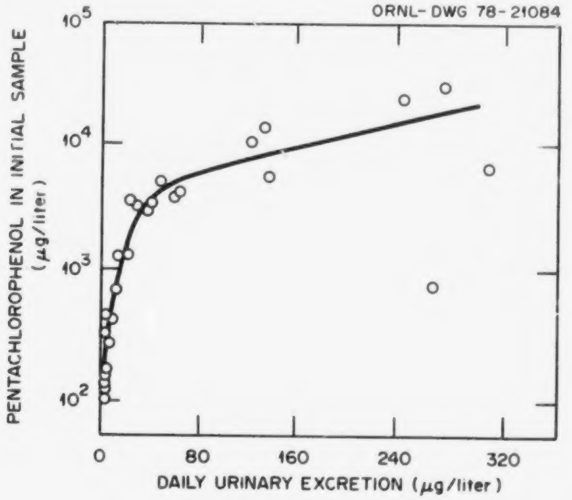

Figure D.6.17. Excretion of orally administered [^{14}C]pentachlorophenol by rats. Source: Compiled from Larsen et al., 1972, Table I, p. 2004.

TABLE D.6.16. PHARMACOKINETICS OF PENTACHLOROPHENOL IN RATS

Sex	Dose (mg/kg)	Pentachlorophenol excreted in 8-9 days (%)		Half-life (hr)		Volume of distribution (ml/kg)
		Urine	Feces	Rapid phase	Slower phase	
Male	10	80.1	18.8	17	40	136
	100	72	24	13	121	NR[a]
Female	10	78.13	19.3	13	33	127
	100	54	43	27[b]	NR	NR

[a] NR — Not reported.
[b] Data were described by a one-compartment open model.

Source: Compiled from Braun et al., 1977.

excretion in the calculations. As pointed out above, the 102-day component could have resulted from the methods used.

As far as can be determined, pentachlorophenol and its sodium salt are excreted with the same kinetics, but some evidence suggests that excretion rates may depend on the route of administration of the compound. Work by Ahlborg, Lindgren, and Mercier (1974) indicates that in rats and mice excretion is higher for intraperitoneal administration of pentachlorophenol than for oral administration (Figure D.6.18). From this research, excretion appears to be more rapid in rats than mice. In general, similar urinary excretion rates are found for rats, rabbits, and mice. Approximately 50% of the administered pentachlorophenol is excreted in the urine in 24 hr and 70% to 85% in four days (Deichmann et al., 1942; Jakobson and Yllner, 1971; Larsen et al., 1972).

The monkey eliminates pentachlorophenol somewhat more slowly than other animals studied. In an experiment by Braun and Sauerhoff (1976), two monkeys were administered a single dose of 10 mg/kg pentachlorophenol. After 360 hr, 70% of the dose was eliminated in the urine, 18% in the feces, and 11% remained in the carcass. Excretion by the kidney was a first-order process characterized by half-lives of 40.8 hr (male) and 92.4 hr (female). Plasma levels decreased by a first-order process with half-lives of 72 hr (male) and 83.5 hr (female).

Although the primary route of pentachlorophenol elimination appears to be urinary, fecal excretion also plays a role. Values ranging from 4% of the injected dose in the rabbit (Deichmann et al., 1942) to 13.2% in rats (Larsen et al., 1972) over a ten-day period have been reported. However, quantitative fecal excretion rates were not readily found in the literature.

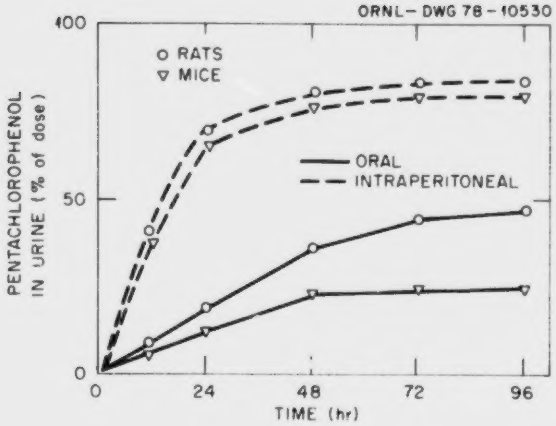

Figure D.6.18. Cumulative excretion of [^{14}C]pentachlorophenol in the urine of rats and mice after a single oral or intraperitoneal dose. Source: Adapted from Ahlborg, Lindgren, and Mercier, 1974, Figure 1, p. 276. Reprinted by permission of the publisher.

It is difficult to draw conclusions concerning the elimination of pentachlorophenol from most of the reported data on urinary excretion in humans for the following reasons: (1) the exposures were accidental or occupational with the quantity unknown, (2) excretion via the fecal route was not determined, and (3) the reports do not account for continued background exposure. There is some speculation that there may be some long-term tissue storage of pentachlorophenol. This hypothesis results from consideration of the long-term storage of chlorinated hydrocarbon insecticides such as DDT and dieldrin in fat; the inference is made that pentachlorophenol may act accordingly. Another factor generating this speculation is data from occupationally exposed individuals whose urine or blood pentachlorophenol levels do not return to zero. Control populations studied also have had low levels in blood and urine; thus, it is possible that continuous low-level exposure may account for these findings.

In future studies on the kinetics of pentachlorophenol the following precautions should be observed:

1. If nonradiotracer techniques are used, background pentachlorophenol levels in water and diet should be monitored, especially in studies dealing with parts-per-billion residue levels.

2. Studies should account for fecal excretion, regardless of the route of exposure.

3. Some plasma protein binding with a specific high affinity, as suggested by Braun et al. (1977), could account for a long-term, low-level residue in blood.

D.6.2 EFFECTS

D.6.2.1 Physiological or Biochemical Role

No normal physiological or biochemical role of pentachlorophenol in humans has been documented. In the absence of a physiological requirement, deficiency syndromes do not occur. Concentrations of pentachlorophenol in tissues and organs reflect exposure of the individual to the compound in some manner.

D.6.2.2 Toxicity

D.6.2.2.1 Mechanisms of Action — Pentachlorophenol is a broad-spectrum biocide. Weinbach (1957) proposed that the mechanism of pentachlorophenol toxicity was either nonspecific, involving a number of unrelated metabolic processes, or — the preferred explanation — involved a basic mechanism common to many forms of life. The primary effect of pentachlorophenol on organisms is likely the uncoupling of oxidative phosphorylation, thereby short-circuiting the energy balance.

On the molecular level, the major effects of pentachlorophenol appear to be on mitochondria and their associated energy systems. The in vitro effects of pentachlorophenol on mammalian or molluscan mitochondria include uncoupling of oxidative phosphorylation, inhibition of mitochondrial and myosin adenosine triphosphatase (ATPase), inhibition of glycolytic phosphorylation, inactivation of respiratory enzymes, and gross damage to mitochondrial structure (Weinbach, 1957). The effects of pentachlorophenol on snail tissue and rat liver at various concentrations in vitro and the possible physiological significance are outlined in Table D.6.17. The systemic effects of pentachlorophenol intoxication can be quite drastic, including changes in and degeneration of many of the vital organs (Section D.6.2.2.2). General systemic effects of pentachlorophenol can be explained on the basis of the basic biochemical alterations described by Weinbach (1957). Table D.6.17 shows that the three ranges of pentachlorophenol concentrations have different effects at the biochemical level. Low concentrations from 10^{-6} to 10^{-4} M uncouple oxidative phosphorylation and result in an increase in respiration. Values of 10^{-3} M and higher drastically curtail glycolytic phosphorylation and result in inactivation of the respiratory enzymes with a drastic reduction in respiration. Intermediate values from 10^{-4} to 10^{-3} M inhibit the action of mitochondrial ATPase and myosin ATPase. Investigations by Mitsuda, Murakami, and Kawai (1963) confirmed these findings and indicated that at intermediate levels of pentachlorophenol, respiration is neither stimulated nor inhibited. The actual process involved in oxidative phosphorylation remains a subject of controversy; thus, a detailed discussion of the action of pentachlorophenol as an uncoupler is not attempted. It is possible, however, from the data available to speculate on the action of pentachlorophenol on oxidative phosphorylation.

Weinbach and Garbus (1965) presented convincing evidence that pentachlorophenol binds preferentially to mitochondrial protein, thereby inducing changes in the enzymes involved with oxidative phosphorylation and

TABLE D.6.17. EFFECTS OF VARIOUS CONCENTRATIONS OF PENTACHLOROPHENOL ON SNAIL TISSUE AND RAT LIVER IN VITRO

Concentration of pentachlorophenol (M)	Effect	Possible physiological significance
10^{-6} to 10^{-4}	Uncoupling of oxidative phosphorylation	Interference with cellular aerobic exergonic processes
10^{-4} to 10^{-3}	Inhibition of mitochondrial ATPase	Unknown
	Inhibition of myosin ATPase	Interference with phosphate transfer (and muscle function?)
10^{-3} and higher	Inhibition of glycolytic phosphorylation Inactivation of respiratory enzymes Gross damage to mitochondrial structure	Rapid death of the cell and of the organism

Source: Adapted from Weinbach, 1957, Table 3, p. 396. Reprinted by permission of the publisher.

preventing the binding or rebinding of functional factors essential for bioenergetic processes. They found that the insoluble protein residue which remained after extraction of lipids and water-soluble components from mitochondria had an affinity for pentachlorophenol equal to or greater than that of intact mitochondria. Weinbach and Garbus (1965) suggested that the interaction of pentachlorophenol with the protein moiety of intact mitochondria is responsible for its uncoupling action.

Further evidence regarding the binding of pentachlorophenol to enzymes was developed by Bowen, Martin, and Jacobus (1965), who utilized actomyosin. Because pentachlorophenol is known to interfere with the enzymatic activity of myosin ATPase, it is reasonable to assume that pentachlorophenol binding plays a role in enzyme inhibition. Acetylation of actomyosin greatly reduced the binding of pentachlorophenol to the muscle protein, suggesting that the amino groups of the protein are the sites of pentachlorophenol binding, probably to lysine and arginine residues.

Hanstein and Hatefi (1974) provided additional support for the proposal that pentachlorophenol binding to mitochondrial proteins is necessary for its uncoupling action. Mitochondrial uncoupler sites were located and characterized by using an uncoupler capable of photoaffinity labeling in the membrane of the mitochondria. Preliminary results indicated that the protein involved in uncoupling had a molecular weight of 20,000 to 30,000. 2,4-Dinitrophenol and sodium azide, two other potent uncouplers, also are apparently bound to the same site as pentachlorophenol. Thus, sound evidence exists for the binding of pentachlorophenol to a mitochondrial protein as a requirement for the uncoupling activity of the substance.

A conflicting view on the role of pentachlorophenol binding to proteins was presented by Stockdale and Selwyn (1971). The kinetics of inhibition of several enzymes by pentachlorophenol was determined and then compared with the kinetics involved in the uncoupling action of pentachlorophenol. They found that pentachlorophenol inhibits several enzymes at concentrations similar to those producing inhibition of soluble mitochondrial ATPase (Table D.6.18). According to Stockdale and Selwyn (1971), "The observations reported here suggest that sensitivity to inhibition by low concentrations of phenols is a fairly general property of kinases and dehydrogenases. The effects of phenols on the ATPase share several features with their effects on other enzymes but none with their effects as uncouplers. No evidence has been found to support the view that the effects on the ATPase are related to uncoupling. For the same reasons these observations do not support the less

TABLE D.6.18. ENZYME INHIBITION BY PENTACHLOROPHENOL

Enzyme	Effect of pentachlorophenol		
	Log 1/I_{50} [a]	Log 1/C_{50} [b]	Log 1/C_m [c]
Hexokinase	2.98		
Pyruvate kinase	3.46		
Creatine phosphokinase	2.70		
Phosphofructokinase	3.90		
Lactate dehydrogenase	3.70		
Malate dehydrogenase	3.96		
Alcohol dehydrogenase	3.89		
Glyoxalate reductase	3.09		
Catalase	<1.52[d]		
Acid phosphatase	<1.70[d]		
ATPase		3.87	4.82

[a] I_{50} – Pentachlorophenol concentration producing 50% inhibition, expressed in molarity.

[b] C_{50} – Pentachlorophenol concentration required to inhibit the enzyme to a rate equal to 50% of the basal rate.

[c] C_m – Pentachlorophenol concentration required to produce maximal stimulation of isolated enzyme.

[d] No significant inhibition observed at this concentration.

Source: Adapted from Stockdale and Selwyn, 1971, Table 1, p. 418. Reprinted by permission of the publisher.

specific suggestion of Weinbach and Garbus (1965) that the interaction of uncouplers with proteins is related to their uncoupling activity." Stockdale and Selwyn (1971) stated that pentachlorophenol has a dual effect on mitochondria, which includes a discrete action in uncoupling oxidative phosphorylation as distinct from the action of pentachlorophenol in inactivating mitochondrial ATPase as well as other enzymes in the cell. Evidence was presented to justify the belief that pentachlorophenol may bind to the active site of the enzyme which normally binds the adenine group in the usual substrate.

It is difficult to reconcile the data of Weinbach and Garbus (1965) with that of Stockdale and Selwyn (1971) unless one proposes a dual action for pentachlorophenol in mitochondria. This dual action includes binding of pentachlorophenol at low concentrations to the protein involved in oxidative phosphorylation. At higher concentrations, however, pentachlorophenol binds to other enzymes in the cell, including mitochondrial ATPase and the respiratory enzymes. This second action might well involve competitive or noncompetitive binding of pentachlorophenol to the active site of the enzymes, thereby displacing the adenine moiety present in the normal substrate. Some caution should be used in interpreting the data of Stockdale and Selwyn (1971), who used purified enzymes in their assay system in contrast to other investigators utilizing intact mitochondria. Some of the conflicting findings may result from permeability effects in the intact mitochondria.

At high levels of pentachlorophenol other indirect effects are possible (Ishak, Sharaf, and Mohamed, 1972). The inhibition of succinate oxidation, which is frequently seen in the presence of uncouplers, may well be due to the accumulation of oxaloacetate in the cell. The presence of high levels of oxaloacetate in the tissue can result in feedback inhibition of succinate dehydrogenase and prevention of succinate oxidation. Effects of this type might result from initial enzyme inactivation caused by high levels of pentachlorophenol in the tissue. Total breakdown of mitochondrial integrity was also reported and could be the final stage of pentachlorophenol toxicity in the cell.

D.6.2.2.2 Local and Systemic Pathology — Both pentachlorophenol and sodium pentachlorophenate can cause local inflammation of the skin on contact. In experiments with rabbits, pentachlorophenol caused irritation of the skin with marked local damage, the intensity of which varied with different solvents (Deichmann et al., 1942). Solutions in petroleum solvents were more likely to cause injury than those in vegetable oils. A 10% solution of sodium pentachlorophenate in water caused edema, inflammation, excoriation, and desquamation of the skin of rabbits receiving a single application (Boyd et al., 1940). Cutaneous application of a 1% aqueous solution of sodium pentachlorophenate to the skin of rabbits for 100 consecutive days resulted in minor effects (Flickinger, 1971). The treated areas occasionally showed minor irritation, but this was not accompanied by wrinkling or cracking of the skin or loss of hair. Solid sodium pentachlorophenate causes more extensive local damage than solid pentachlorophenol. Marked irritation or even burns follow prolonged contact with sodium pentachlorophenate (Dow Chemical Company, 1969*a*).

Prolonged skin contact with pentachlorophenol solid results in only slight redness (Dow Chemical Company, 1969*b*). Skin irritation may develop after seemingly small exposures to pentachlorophenol in solution. In one case, immersion of hands for 10 min in a 0.4% solution of pentachlorophenol caused severe pain and inflammation (Bevenue, Haley, and Klemmer, 1967).

A serious skin disease known as chloracne can result from prolonged cutaneous contact with technical pentachlorophenol or sodium pentachlorophenate. The disease is characterized by the formation of comedones with or without cysts and pustules (Kimbrough, 1972) and is due to chlorodioxin contaminants. The follicular orifices are filled with sebaceous and keratinous material. Melanosis and a secondary inflammatory reaction may occur. Chloracne is one of the most frequent forms of occupational dermatitis, and many cases have been reported in the United States and Europe, particularly Germany and England. Behrbohm (1959) gave a detailed account of about 100 cases of skin diseases and irritations (chloracne and dermatitis) resulting from the use of sodium pentachlorophenate as a wood preservative. Severe cases of chloracne and eczematous skin lesions among workers engaged in the production of technical pentachlorophenol have been reported by Baader and Bauer (1951) and Nomura (1953). Ten percent solutions of sodium pentachlorophenate in water and pentachlorophenol in chloroform produced chloracne responses in rabbits (Dow Chemical Company, 1969*a*, 1969*b*).

The dust, mist, or vapor from heated materials or solutions and direct contact of solutions with the eyes causes irritation (American Industrial Hygiene Association, 1970). Pentachlorophenol dust applied to rabbit eyes caused slight pain and conjunctival redness and, in one of four animals, slight iritis (Dow Chemical Company, 1969*a*). Application of undiluted Dowicide G (sodium pentachlorophenate) caused marked pain, moderate to severe conjuctival irritation, severe corneal damage, and moderate iritis; only minor healing was observed in seven days (Dow Chemical Company, 1969*b*).

Concentrations of these compounds in dust and mist greater than 1.0 mg/m^3 caused painful irritation in the upper respiratory tract of persons newly exposed to pentachlorophenol, accompanied by violent sneezing and coughing. Concentrations as high as 2.4 mg/m^3 can be tolerated by those conditioned to exposures (American Industrial Hygiene Association, 1970).

Autopsies of victims of pentachlorophenol poisoning have shown moderate to gross pathological changes of the gastrointestinal tract, lungs, spleen, liver, kidneys, and cardiovascular system. The systemic effects of acute pentachlorophenol poisoning in animals closely mimic the effects in humans. Pentachlorophenol apparently does not have the convulsive action of phenol. According to Kehoe, Deichmann-Gruebler, and Kitzmiller (1939), Boyd et al. (1940), McGavack et al. (1941), and Deichmann et al. (1942), it produces the following toxicological picture. A period of general depression is followed by an increase in respiratory rate and volume. The pulse rate increases and is associated with a primary rise and subsequent fall of the blood pressure (in the absence of electrocardiographic changes); neuromuscular weakness develops, especially towards the end, with a rise of the body temperature. At the time of the rise of body temperature, the blood sugar level is increased and sugar is excreted in the urine, which, according to McGavack et al. (1941), results in depletion of the glycogen content of the liver so that the final convulsive seizures may be due to acute hypoglycemia. In addition, increased peristalsis and thirst, diuresis, and later, oliguria occur. In fatal poisonings the animals die from heart failure. Boyd et al. (1940) suggested that the variations in blood sugar of intoxicated animals may result from pathological changes in the liver caused by pentachlorophenol and a gradual breakdown of the processes by which the liver mobilizes carbohydrates and fats. The pathological symptoms mentioned above have been noted in experiments on rabbits, dogs, rats, and guinea pigs, and similar pathological changes have been observed in human autopsies.

Good data regarding the mechanisms of these pathological changes in various organs are not available. Probably of greatest importance is the uncoupling of oxidative phosphorylation, thereby causing severe metabolic disturbances in tissues where pentachlorophenol reaches a critical threshold. The general hyperpyrexia caused by pentachlorophenol may be the indirect cause of many of the pathological changes. Human autopsy results, as well as data from experimental animals, are discussed below according to the type of pathology seen in specific organs.

D.6.2.2.2.1 Gastrointestinal tract — Inflamed gastric mucosa and acute gastritis were noted in fatal cases of pentachlorophenol intoxication by Menon (1958). He suggested that the presence of these pathological changes were due to entry of pentachlorophenol into the body via the gastrointestinal route. Pathological changes of the gastrointestinal tract are not a common finding in pentachlorophenol poisoning; therefore, the changes noted were likely due to entry of pentachlorophenol via the oral route.

D.6.2.2.2.2 Lungs — Pathological changes of the lungs following fatal pentachlorophenol intoxication have been commonly reported. Congestion of the lungs was reported in two fatal cases of pentachlorophenol poisoning by Blair (1961). Edematous lungs and gross pulmonary congestion in fatal cases of pentachlorophenol poisoning were reported by Gordon (1956), Menon (1958), Mason et al. (1965), and Bergner, Constantinidis, and Martin (1965). Menon (1958) suggested that congestion and edema of the lungs resulted from uptake via the respiratory route. However, congestion of the lungs and alveoli in cases where the respiratory tract was not an important route of uptake suggests that a more general pathological condition may exist in the lungs following pentachlorophenol intoxication. This congestion could be due to general damage to the cardiovascular system, especially the small vessels (Kehoe, Deichmann-Gruebler, and Kitzmiller, 1939; Boyd et al., 1940).

D.6.2.2.2.3 Spleen — Splenomegaly was reported in three cases of fatal pentachlorophenol intoxication by Menon (1958) and Robson et al. (1969).

D.6.2.2.2.4 Liver — Hepatomegaly, congestion, centrilobular degeneration of liver cells, and general fatty degeneration of liver tissue were reported in autopsies of people who died from pentachlorophenol intoxication (Gordon, 1956; Bergner et al., 1965; Mason et al., 1965; Robson et al., 1969). As mentioned before, the hyperglycemia and glycosuria often noted in investigations of experimental animals may be due to a direct pathological effect of pentachlorophenol on the liver. Gross hepatomegaly and general liver damage have been frequently reported when pentachlorophenol is administered to experimental animals. Changes in the activity of microsomal liver enzymes were reported by Knudsen et al. (1974) and Goldstein et al. (1977) when pentachlorophenol was included in the diet of rats (Table D.6.19). In serious cases of pentachlorophenol poisoning, Nomura (1953) noted a decrease in the albumin to globulin ratio and urinary excretion of urobilinogen, suggesting the presence of some liver lesions.

D.6.2.2.2.5 Kidneys — Kidney damage commonly results from pentachlorophenol intoxication. Hydropic and fatty degeneration of renal tubules and general congestion were reported by Bergner, Constantinidis, and Martin (1965), Mason et al. (1965), and Robson et al. (1969). A common finding in both fatal and nonfatal human intoxications is the presence of metabolic acidosis, proteinuria, and elevated urea nitrogen in the blood (Chapman and Robson, 1965). These changes in blood chemistry may be due to the effect of pentachlorophenol on kidney tissue.

D.6.2.2.2.6 Heart — Cardiomegaly and fatty degeneration of the myocardium were reported by Robson et al. (1969).

D.6.2.2.2.7 Aplastic anemia — A case of aplastic anemia due to pentachlorophenol and tetrachlorophenol was reported by Roberts (1963). The case involved a 21-year-old truck driver who handled lumber soaked in a product containing pentachlorophenol and tetrachlorophenol. His clothing and hands frequently became drenched, and some oral contamination probably occurred because he was a habitual nail biter. Postmortem examination failed to reveal any other causes for the aplastic anemia; therefore, it was concluded that the damage to the bone marrow was caused by exposure to the two chemicals.

D.6.2.2.2.8 Peripheral neuropathy — Although it has frequently been noted that pentachlorophenol can cause peripheral neuropathy, a critical examination of the literature reveals that the cases of peripheral neuropathy reported after exposure to pentachlorophenol are unclear. Neuropathy is not part of the usual picture of pentachlorophenol poisoning. One man, however, developed various peripheral nerve palsies after gross overexposure of his arms to pentachlorophenol (Fullerton, 1969). Seven cases of peripheral neuritis were linked to the use of an insecticide mixture containing pentachlorophenol (Campbell, 1952). The exact formulation of the insecticide was unknown, but it contained *o*- and *p*-dichlorobenzene, DDT, and pentachlorophenol. The presence of several substances makes it impossible to reach a definite conclusion as to the causative agent.

TABLE D.6.19. ACTIVITY OF MICROSOMAL LIVER ENZYMES IN RATS MAINTAINED ON DIETS CONTAINING PENTACHLOROPHENOL FOR 12 WEEKS

Number and sex of rats	Week of experiment	Enzyme	Enzyme activity at various levels of pentachlorophenol in diet (micromoles/liter)[a]			
			0 mg/kg	25 mg/kg	50 mg/kg	200 mg/kg
6 males	6	Aniline hydroxylase	163 ± 45	170 ± 46	202 ± 44	261 ± 54[b]
		Aminopyrine demethylase	19 ± 7	17 ± 11	30 ± 15	33 ± 11[c]
		Glucose-6-phosphatase[d]	92 ± 38	90 ± 30	102 ± 36	79 ± 18
4 males	12	Aniline hydroxylase	196 ± 42	222 ± 28	281 ± 58	410 ± 58[e]
		Aminopyrine demethylase	36 ± 5	34 ± 13	44 ± 8	52 ± 17
		Glucose-6-phosphatase[d]	148 ± 22	144 ± 10	130 ± 19	116 ± 35
4 females	12	Aniline hydroxylase	264 ± 45	249 ± 53	290 ± 37	391 ± 46[c]
		Aminopyrine demethylase	23 ± 6	17 ± 4	18 ± 7	21 ± 6
		Glucose-6-phosphatase[d]	93 ± 11	100 ± 7	127 ± 26	104 ± 14

[a] Average ± standard deviation.

[b] $P < 0.01$.

[c] $P < 0.05$.

[d] Activity expressed as units per milliliter. A unit is the amount of enzyme needed to catalyze the transformation of 1 micromole substrate per minute.

[e] $P < 0.001$.

Source: Adapted from Knudsen et al., 1974, Table IV, p. 146. Reprinted by permission of the publisher.

Another case of peripheral neuritis was reported by Wagle (1974). The use of an antitermite formulation resulted in the stiffening of finger joints with partial incapacitation of both hands. The formulation was found to contain dieldrin (0.5%), pentachlorophenol (1%), tar acids (1%), and a xylene-kerosene (1:9) solvent base. As in the previous case, the presence of other substances prevents ascribing this neuritis to pentachlorophenol. The question of whether or not pentachlorophenol causes peripheral neuritis remains an open one. It seems, however, that if pentachlorophenol were a significant causative agent in the development of peripheral neuritis, many more cases would have been reported over the time span in which pentachlorophenol has been used.

D.6.2.2.3 Acute Toxicity — Dose-response curves are difficult, if not impossible, to determine accurately with human data because varying exposure conditions permit only estimates of actual doses. Whenever possible, data from experimental animals are extrapolated to humans.

Absorption of pentachlorophenol in amounts sufficient to cause systemic intoxication can occur by the oral, respiratory, or cutaneous routes, and, of course, occupationally exposed persons are subject to the greatest hazard. Although members of the general population exhibit a low level of pentachlorophenol contamination (Bevenue et al., 1967; Cranmer and Freal, 1970), they do not have symptoms found in occupationally exposed individuals. People poisoned by acute or chronic high-level doses show similar symptoms: weight loss, general weakness, fatigue, dizziness, mental weakness, headache, anorexia, nausea and vomiting, dyspnea, hyperpyrexia, respiratory distress, tachycardia, hepatomegaly, profuse perspiration, and an elevated basal metabolic rate (Nomura, 1953; Truhaut, Boussemart, and L'Épée, 1952; Bergner, Constantinidis, and Martin, 1965; Robson et al., 1969). These symptoms have been found in both fatal and nonfatal intoxications. In acute cases, onset of symptoms may be very rapid, with death ensuing in 3 to 30 hr. The most common symptoms are general weakness, weight loss (in chronic cases), and profuse perspiration. The clothes of one individual admitted to a hospital in a semicomatose condition following exposure to pentachlorophenol were so wet that the admitting examiners incorrectly surmised that he had been dragged from a nearby river. The cause of fever and profuse sweating is the uncoupling of oxidative phosphorylation accompanied by the equivalent of tissue hypoxia. The hyperpyrexia itself may be the primary factor in bringing about circulatory and respiratory failure by virtue of the central and peripheral effects of the fever, as in the case of heat stroke (Mason et al., 1965).

Only about 51 cases of poisoning from pentachlorophenol ingestion or absorption have been reported, but 30 of those resulted in death. No specific treatment for the poison is known, and the deaths took place in spite of the conventional supportive therapy (Anonymous, 1970). Successful treatment of pentachlorophenol poisoning was reported by Robson et al. (1969) in a case of epidemic pentachlorophenol poisoning among infants in a St. Louis nursery. Sodium pentachlorophenate in diapers and hospital linens was absorbed cutaneously by the infants.

Nine infants between the ages of 6 and 14 days were severely affected. The course of the illness was rapid, with progressive deterioration. One infant died within 3 hr following onset of the first symptom; another died before she could be given an exchange transfusion. Of the seven who had severe symptoms, six underwent exchange blood transfusions (the same treatment used in cases of erythroblastosis). They improved promptly and survived. Eleven other children who showed less serious forms of the illness survived without any active treatment. Most cases of pentachlorophenol poisoning are in adults exposed to large quantities of the chemical. These infants were repeatedly exposed to relatively small quantities in fabrics. Armstrong et al. (1969) suggested that the increased susceptibility shown by these infants may have resulted from their immature renal functions. Nomura (1953) also suggested that susceptibility to pentachlorophenol poisoning may depend on ability to excrete pentachlorophenol. Truhaut, Boussemart, and L'Épée (1952) demonstrated that nephritic rabbits were more sensitive to pentachlorophenol than normal animals.

Relatively high pentachlorophenol levels in the blood and urine of workers involved with wood preservatives are numerous (Casarett et al., 1969). Some concentrations reported for blood and urine in cases of acute pentachlorophenol intoxications are no greater than two to five times the high values reported for apparently healthy workers involved in timber dipping operations. Urinary levels of pentachlorophenol reported in fatal and nonfatal cases (Table D.6.20) show that levels in fatal and nonfatal poisonings overlap to a considerable extent. Thus, the margin between fatal and nonfatal pentachlorophenol poisoning and apparent good health may be extremely narrow. The exact dosage of pentachlorophenol required to produce illness in humans is not known. Symptoms occur at concentrations of 40 to 80 mg/liter in the blood. Pentachlorophenol is excreted in the urine at levels of 3 to 60 mg/liter in nonfatal cases. Blood levels noted in fatal cases ranged from 46 to 156 mg/liter and urine levels from 28 to 520 mg/liter. The *Registry of Toxic Effects of Chemical Substances* (Christensen and Luginbyhl, 1975) gives the oral lethal dose of pentachlorophenol as 29 mg/kg body weight. Based on this figure, a 150-lb man would have to absorb nearly 2 g of pentachlorophenol of sodium pentachlorophenate before it would be lethal. As noted previously, for persons whose ability to excrete the compound is hindered in some way, the lethal dose may be considerably lower (Armstrong et al., 1969).

The lethal doses of sodium pentachlorophenate and pentachlorophenol administered via the oral and cutaneous routes to experimental animals are given in Table D.6.21. The lethal oral dose of pentachlorophenol ranges from 27 to 205 mg/kg body weight in rats, rabbits, and guinea pigs. The 27 mg/kg dose was administered to the rat in fuel oil. The contribution of the fuel oil to the lethality is unknown. The cutaneous lethal doses ranged from 100 to 200 mg/kg body weight in rabbits. Similar values were found for sodium pentachlorophenate tested in experimental animals. The oral acute dose in rats, rabbits, and guinea pigs is 80 to 274 mg/kg body weight, and the cutaneous acute dose is 100 to 300 mg/kg body weight. The toxicity of pentachlorophenol depends on the solvent in which it is applied. The varying values mentioned above are for various solvents

TABLE D.6.20. PENTACHLOROPHENOL LEVELS IN URINE OF FATALLY AND NONFATALLY POISONED HUMANS

Compound	Individual and occupation	Pentachlorophenol in urine (mg/liter)	Source
	Fatally poisoned		
Sodium pentachlorophenate	22-year-old male, dipping planks in preservative	160	Menon, 1958
	16-year-old male, spraying pineapples	70	Gordon, 1956
	14-year-old male, spraying watercourses for snail control	28	Blair, 1961
	Male, bagging pentachlorophenol at chemical factory	520	Mason et al., 1965
	Nonfatally poisoned		
Pentachlorophenol	3-year-old female, accidental	60	Chapman and Robson, 1965
	36-year-old male, dipping planks	18	Bergner, Constantinidis, and Martin, 1965
	21-year-old male, dipping planks	3.8	Bergner, Constantinidis, and Martin, 1965
	38-year-old male, dipping planks	5.4	Bergner, Constantinidis, and Martin, 1965
	30-year-old male, dipping planks	10	Bergner, Constantinidis, and Martin, 1965

TABLE D.6.21. LETHAL DOSES OF PENTACHLOROPHENOL AND SODIUM PENTACHLOROPHENATE ADMINISTERED ORALLY AND CUTANEOUSLY TO RATS, RABBITS, AND GUINEA PIGS

Route of administration	Solvent	Animal	Dose (mg/kg body wt)	Source
		Pentachlorophenol		
Oral	11% in olive oil	Rabbit	100-130^a	McGavack et al., 1941
	5% in fuel oil	Rabbit	70-90^a	Deichmann et al., 1942
	Not known	Rat, male	205	Dow Chemical Company, 1969*a*
		Rat, female	135	Dow Chemical Company, 1969*a*
	1% in olive oil	Rat	78^b	Deichmann et al., 1942
	0.5% in fuel oil	Rat	27^b	Deichmann et al., 1942
Cutaneous	Various, not known	Rabbit	40-170^a	Deichmann et al., 1942
	Not known	Rabbit	100-200	Dow Chemical Company, 1969*a*
		Sodium pentachlorophenate		
Oral	Aqueous	Rabbit	218^a	Kehoe, Deichmann-Gruebler, and Kitzmiller, 1939
	Aqueous	Rabbit	275	Dow Chemical Company, 1969*b*
	Aqueous	Rabbit	250-300^a	Deichmann et al., 1942
	Aqueous	Rat	210	Dow Chemical Company, 1969*b*
	Aqueous	Rat	211	Deichmann et al., 1942
	Aqueous	Guinea pig	80-160	Dow Chemical Company, 1969*b*
Cutaneous	Aqueous	Rabbit	250^a	Deichmann et al., 1942
	Aqueous	Rabbit	450-600^a	McGavack et al., 1941
	Aqueous	Rabbit	100-300	Dow Chemical Company, 1969*b*
	Aqueous	Guinea pig	266^a	Kehoe, Deichmann-Gruebler, and Kitzmiller, 1939

[a] Minimum lethal dose.

[b] LD_{50} value.

under various conditions. The dose-response relationship of sodium pentachlorophenate cutaneously applied to rabbits is given in Table D.6.22. Pentachlorophenol appears to be more toxic to mammals than sodium pentachlorophenate, at least when the route of administration is oral or cutaneous (Table D.6.23). With subcutaneous administration the difference in toxicity disappears. Differential absorption may explain this phenomenon.

The toxicity of pentachlorophenol or sodium pentachlorophenate may be influenced by three factors: (1) ambient temperature, (2) renal competency, and (3) general health. Most cases of fatal pentachlorophenol poisoning have occurred when ambient temperatures were high — approximately 27°C to 33°C. The increased volatility of pentachlorophenol at higher temperatures may increase the amount absorbed by inhalation. Because a primary effect of pentachlorophenol intoxication is hyperpyrexia, the increased toxicity of pentachlorophenol whem ambient temperatures are high may be due to increased difficulty in dissipating the excess heat generated by the uncoupling of oxidative phosphorylation.

The renal competency of pentachlorophenol-intoxicated persons determines their tolerance to exposure. Deichmann et al. (1942) found that tolerance developed in rabbits when sublethal oral doses of pentachlorophenol were repeatedly administered. Such a tolerance effect has not been demonstrated in humans. However, a tolerance to the respiratory distress caused by high atmospheric levels of pentachlorophenol occurs in occupationally exposed workers as distinct from a general systemic tolerance to the compound.

A third factor which may influence the toxicity of pentachlorophenol to man is general health (Menon, 1958). All nine deaths from pentachlorophenol poisoning in Sarawak, Borneo, were members of true native races (e.g., Dyak and Kayan) rather than the Chinese or Malays, even though as many, if not more, of these nonnative races were working on the same job. The native races had average heights of 5 ft 4 in. and weights of 45 to 50 kg, and most were suffering from mixed worm infestation and malaria. In almost every case their diet was rice with little or no protein. The general poor health and physique of these people probably enhanced the toxic responses seen following exposure to pentachlorophenol.

D.6.2.2.4 Chronic Toxicity

D.6.2.2.4.1 In experimental animals — The effects of chronic levels of pentachlorophenol administered to experimental animals are difficult to determine. Attempts to produce high-dose chronic intoxications via the cutaneous route have been difficult because of the severe local reactions. Low-dose, chronic systemic intoxications, in general, have not been shown via the cutaneous route. Cutaneous application of pentachlorophenol at levels low enough to avoid gross skin damage resulted in no chronic systemic disease in rabbits (Kehoe, Deichmann-Gruebler, and Kitzmiller, 1939).

Table D.6.24 shows the results of daily administering subcutaneous or intraperitoneal doses of sodium pentachlorophenate to rabbits.

TABLE D.6.22. CLINICAL EFFECTS OF CUTANEOUS ADMINISTRATION OF SODIUM PENTACHLOROPHENATE TO RABBITS

Dose (mg/kg)	Average temperature 4 hr after dose[a] (°C)	Average blood sugar 4 hr after dose (mg/100 ml)	Number of defecations during 4 hr	Died (%)
50			2	0
100	39.8	141	3	0
150	40.2		8	0
200	40.1		7	0
250	39.6		7	0
300	40.3		9	33
350	40.6	256	7	0
400			4	0
450	40.6			50
500	39.4	238	9	100
550	40.6		8	50
600	42.4	243	17	100

[a] Normal body temperature is approximately 39.4°C.

Source: Adapted from Boyd et al., 1940, Table I, p. 324. Reprinted by permission of the publisher.

TABLE D.6.23. COMPARISON OF THE TOXICITIES OF PENTACHLOROPHENOL AND SODIUM PENTACHLOROPHENATE TO RABBITS

Route of administration	Compound and solvent	Lethal dose (mg/kg)
Cutaneous	5% pentachlorophenol in Stanolex fuel oil	60-70
	10% sodium pentachlorophenate in water	250
Oral	5% pentachlorophenol in Stanolex fuel oil	70-90
	10% sodium pentachlorophenate in water	250-300
Subcutaneous	5% pentachlorophenol in olive oil	70-85
	10% sodium pentachlorophenate in water	100

Source: Adapted from Deichmann et al., 1942, Table 1, p. 106. Reprinted by permission of the publisher.

TABLE D.6.24. CHRONIC TOXICITY OF SODIUM PENTACHLOROPHENATE TO RABBITS

Number of rabbits	Route of administration	Daily dose (mg/kg)[a]	Average number of injections	Average total dose (mg)	Number of rabbits			
					Died	Decreased weight	Stationary weight	Increased weight
6	Subcutaneous	13.7	52	716	0	3	0	3
6	Subcutaneous	27.5	15	401	6	4	2	0
6	Subcutaneous	70.0	8	516	6	6	0	0
3	Intraperitoneal	15.0	11	165	3	3	0	0

[a] Minimum lethal doses were 150 mg/kg body weight by intraperitoneal injection and 275 mg/kg body weight by subcutaneous application in a single dose.

Source: Adapted from McGavack et al., 1941, Table 3, p. 244. Reprinted by permission of the publisher.

McGavack et al. (1941) found that the amount of sodium pentachlorophenate necessary to cause death in small, divided doses was very close to the minimum lethal dose recorded when a single administration of the compound was studied.

When daily doses of sodium pentachlorophenate were administered to rabbits at a dose rate of 1 mg/kg body weight for 90 days, no systemic effects were found and blood levels of pentachlorophenol were undetectable (Machle, Deichmann, and Thomas, 1943). However, when pentachlorophenol was fed to rabbits at a dose rate of 3 mg/kg body weight over a 90-day period, blood levels of 0.6 mg per 100 ml of blood were reported. No systemic effects were found at these dose levels (Deichmann et al., 1942). These levels of pentachlorophenol in blood (0.6 mg per 100 ml) were approximated closely by skin application of sodium pentachlorophenate at a rate of 100 mg/day for 100 daily doses. This dose schedule resulted in a level of 0.45 mg per 100 ml. For comparative purposes, levels of pentachlorophenol in blood of rats following a lethal dose of pentachlorophenol reportedly ranged from 4.5 to 8.0 mg per 100 ml (Deichmann et al., 1942).

Studies of the effects of chronic ingestion of pentachlorophenol or sodium pentachlorophenate have been complicated by the fact that these substances are repellent to animals. Thus, food intake generally decreases, and loss of weight is seen as a result of the decreased food intake. In such cases, pathological changes in the dosed animals were not seen. If the amount of pentachlorophenol or sodium pentachlorophenate in the diet is reduced to a very small sublethal dose, no chronic systemic disease is apparent. Daily doses of sodium pentachlorophenate administered orally to rabbits at a level of 1 mg/kg body weight for 90 days resulted in no poisoning and only very limited retention of the compound in the blood (Machle, Deichmann, and Thomas, 1943). Daily feeding of sodium pentachlorophenate at a level of 3 mg/kg body weight to rabbits resulted in no signs of acute poisoning but showed a blood accumulation of up to 0.6 mg pentachlorophenol per 100 ml.

Inclusion of pentachlorophenol or sodium pentachlorophenate in the diet of cats resulted in a decreased intake of food (Deichmann et al., 1942). Cats totally refused food containing pentachlorophenol at a level which, with normal intake of food, would result in a dose of 5 to 10 mg/kg body weight. When the pentachlorophenol content was decreased, it was possible to expose the cats to 1.25 to 2.5 mg/kg body weight daily. This diet was fed for ten weeks. Some loss of weight and appetite occurred, and pentachlorophenol levels of 0.3 to 1.8 mg per 100 g of blood were found. Application of pentachlorophenol in mineral oil to the skin of rabbits at a dose rate of 40 mg/kg for 21 consecutive days did not result in illness, weight loss, or skin injury. Application of sodium pentachlorophenate to the skin at a dose level of 40 mg/kg for 100 consecutive days also resulted in a normal weight gain and no fatalities (Deichmann et al., 1942). When 113 mg/kg sodium pentachlorophenate was administered cutaneously, death resulted following two consecutive daily treatments.

Rabbits showed some degree of tolerance when substantial doses of pentachlorophenol were administered over a period of time. Deichmann et al. (1942) administered pentachlorophenol to rabbits at a daily dose rate of 35 mg/kg (12% of the minimum lethal dose) for 15 days. For the following 19 days, daily doses were gradually increased to 600 mg/kg (twice the minimum lethal dose). A loss of weight and a decrease in erythrocyte and hemoglobin counts were noted. Analysis of the blood following death revealed levels of pentachlorophenol ranging from 14 to 39 mg per 100 g. These levels are approximately ten times the levels seen in animals poisoned by a single lethal dose of pentachlorophenol.

Several 90-day or longer studies of chronic pentachlorophenol toxicity have been reported in recent years. Kimbrough and Linder (1975) fed a relatively pure pentachlorophenol (containing low levels of chlorodibenzo-p-dioxin contaminants) to a group of ten male rats and fed commercial pentachlorophenol (containing a relatively high concentration of these contaminants) to a second group for 90 days. A level of 1000 mg pentachlorophenol per kilogram food, equivalent to approximately 50 mg/kg body weight per day, was fed to the rats, and liver changes were examined by light and electron microscopy. Enlargement of hepatocytes was observed in livers of rats fed purified pentachlorophenol; technical grade pentachlorophenol caused foamy cytoplasm, vacuoles, inclusions, single hepatocellular necrosis, slight interstitial fibrosis, and prominent brown pigment in macrophages and Kupffer's cells. Electron microscopic analysis showed an increase in the smooth endoplasmic reticulum of the group fed technical pentachlorophenol and less change in the rats fed purified pentachlorophenol. There were many lipid vacuoles in the former group and some in the latter. Atypical mitochondria were observed in the livers of both groups.

Knudsen et al. (1974) fed weanling Wistar rats diets containing 25, 50, and 200 mg pentachlorophenol per kilogram food (approximately 1.5, 3, and 14 mg/kg body weight respectively) for 12 weeks. Examination of the results in Table D.6.25 shows that a number of the observed changes do not fit a dose-response pattern. For example, female rats fed 25 and 200 mg/kg gained less weight than the controls, but the intermediate group receiving 50 mg/kg did not show this effect. Blood urea increased in the females receiving 25 mg/kg but not in rats fed the two higher doses. Some of these changes could be differences due to chance; the statistical method used was Student's *t* test. More appropriate statistical methods are available for making multiple comparisons of graded treatment levels with a common control group. Hemoglobin values and the number of erythrocytes decreased in groups of male rats fed 50 and 200 mg/kg. In addition to the results shown in Table D.6.25, relative liver weight increased in the females fed 50 and 200 mg/kg. There was an apparent increase in centriobular vacuolization in the livers in these groups; however, vacuolization was also seen in control males. The liver enzyme data are given in more detail in Table D.6.19.

An interesting effect of pentachlorophenol on the kidneys of rats given dietary levels of pentachlorophenol for 12 weeks was reported by Knudsen et al. (1974). The histopathology of liver and kidney tissues

TABLE D.6.25. TOXICITY OF PENTACHLOROPHENOL TO RATS IN A 12-WEEK FEEDING STUDY[a]

Parameter	Effect of pentachlorophenol at various levels in feed[b]					
	Males[c]			Females[c]		
	25 mg/kg	50 mg/kg	200 mg/kg	25 mg/kg	50 mg/kg	200 mg/kg
Feed consumption	0	0	0	0	0	0
Body weight	0	0	0	<	0	<
Biochemistry (12 weeks)						
Serum glutamic-pyruvic transaminase	0	0	0	0	0	0
Alkaline phosphatase	0	0	0	0	0	0
Urea	0	0	0	>	0	>
Glucose	0	0	>	0	0	0
Liver enzymes (12 weeks)						
Aniline hydroxylase	0	0	>	0	0	>
Aminopyrine demethylase	0	0	0	0	0	0
Glucose-6-phosphate	0	<	0	0	0	0
Hematology (11 weeks)						
Hemoglobin	0	<	<	0	0	0
Hematocrit	0	0	0	0	0	0
Erythrocyte count	0	<	<	0	0	0

[a] Technical grade pentachlorophenol with 200 mg/kg octachlorodibenzo-*p*-dioxin and unspecified hexachlorodibenzo-*p*-dioxin content.

[b] Statistical analysis by Student's *t* test. 0 indicates no effect; > indicates an increase; < indicates a decrease.

[c] Ten males and ten females per group.

Source: Compiled from Knudsen et al., 1974.

is shown in Table D.6.26. A dose-related decrease of calcium deposits in the kidney was indicated. Knudsen et al. (1974) suggested that this effect on calcium deposition in the kidney may arise when:

(a) PCP or a metabolite has chelating properties. This does not seem very likely in view of the chemical structure of the parent compound.

(b) Calcium deposits are not formed due to the acidity of the pre-urine. However, the pH of the urine in all groups was 6 when checked in week 11, so no differences were observed between control and test groups. This is evidence against such a possibility, although pH is not measured at other time intervals.

(c) The Ca^{2+}-blood level is lowered through an indirect action of PCP on the calcium metabolism. A number of mechanisms might account for this observation. Normally calcium metabolism depends on the adrenals, parathyroid and pituitary glands. More work is needed to elucidate the reason for the decreased calcium deposits.

Goldstein et al. (1977) fed weanling female rats (Sherman strain) either pure (reagent grade) or technical grade pentachlorophenol for eight months in a study designed to evaluate liver enzyme induction. The rats received 20, 100, and 500 mg pentachlorophenol per kilogram of feed, which is approximately equal to 1.2, 6, and 30 mg/kg body weight respectively. The results are summarized in Table D.6.27. Pure pentachlorophenol was not an inducer of aryl hydrocarbon hydroxylase, aminopyrine-*N*-demethylase, cytochrome P-450, or ALA synthetase. A high dose of pure pentachlorophenol did increase the activity of glucuronyl transferase. Technical pentachlorophenol with dioxin and furan contaminants induced aryl hydrocarbon hydroxylase and glucuronyl transferase at all dosage levels. Cytochrome P-450 activity increased at the two higher dosages. The increase in liver enzymes was accompanied by an increase in absolute and relative liver weight. In addition, about one-third of the rats fed diets containing 100 or 500 mg technical pentachlorophenol per kilogram of food had an increase in total liver porphyrins, mainly uroporphyrins. Neither pure nor technical pentachlorophenol induced aminopyrine-*N*-demethylase activity, which is consistent with the results of Knudsen et al. (1974). It is apparent that the contaminants in technical pentachlorophenol are inducers of several liver enzymes involved in metabolism of chemicals. Additionally, both pure and technical pentachlorophenol decreased the amount of body weight gained during the eight months of feeding. The rats weighed 100 g each at the beginning of the experiment; final weights were 310 g for controls, 270 g for rats fed 500 mg pure pentachlorophenol per kilogram of food, and 245 g for rats fed 500 mg technical pentachlorophenol per kilogram food.

Kociba et al. (1971) fed male, seven-week-old, Sprague-Dawley rats pure or technical pentachlorophenol for 90 days at dosages of 0, 3, 10, and 30 mg/kg body weight (Table D.6.28). There were small depressions

TABLE D.6.26. HISTOPATHOLOGY OF LIVER AND KIDNEY TISSUES FOLLOWING ORAL ADMINISTRATION OF PENTACHLOROPHENOL TO RATS FOR 12 WEEKS

Alteration to organs	Number of rats affected at various levels of pentachlorophenol in food[a]							
	0 mg/kg		25 mg/kg		50 mg/kg		200 mg/kg	
	Males	Females	Males	Females	Males	Females	Males	Females
Liver								
Bile duct proliferation	1	0	0	0	1	1	1	0
Centrilobular vacuolization	0	2	0	0	0	4	2	5
Kidney								
Basophilic proximal tubules	0	0	1	0	0	0	0	0
Some calculi in cortico-medullary junction	0	0	2	0	2	0	3	0
Moderate number of calculi	2	0	4	0	4	0	1	0
Many calculi	6	0	4	0	2	0	1	0
Hydronephrosis	1	0	0	0	0	0	0	0

[a] Ten males and ten females per group.

Source: Adapted from Knudsen et al., 1974, Table VII, p. 149. Reprinted by permission of the publisher.

TABLE D.6.27. HEPATIC EFFECTS OF PURE AND TECHNICAL GRADE PENTACHLOROPHENOL IN FEMALE RATS DURING AN EIGHT-MONTH FEEDING STUDY[a]

Parameter	Effect of pentachlorophenol at various levels in feed[b]					
	Pure[c]			Technical grade[d]		
	20 mg/kg	100 mg/kg	500 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Liver enzymes						
Aryl hydrocarbon hydroxylase	0	0	0	>	>	>
Aminopyrine *N*-demethylase	0	0	0	0	0	0
Cytochrome P-450	0	0	0	0	>	>
Glucuronyl transferase	0	0	>	>	>	>
ALA synthetase	0	0	0	0	0	0
Microsomal heme	0	0	0	0	>	>
Liver/body weight	0	0	0	0	>	>
Absolute liver weight	0	0	0	0	0	>
Coproporphyrin	0	0	0	0	>	>
Uroporphyrin	0	0	0	0	0	0
Urinary ALA	0	0	0	0	>	>
PBG	0	0	0	0	0	0
Body weight	0	0	<	0	0	<

[a] Statistical analysis by analysis of variance followed by Duncan's multiple-range test at $P = 0.05$. Six rats per group.

[b] 0 indicates no significant effect; > indicates a significant increase; < indicates a significant decrease.

[c] From Aldrich Chemical Company; >99% pure.

[d] Contained 8 mg/kg hexachlorodibenzo-*p*-dioxin, 520 mg/kg heptachlorodibenzo-*p*-dioxin, 1380 mg/kg octachlorodibenzo-*p*-dioxin, 4 mg/kg tetrachlorodibenzofuran, 40 mg/kg pentachlorodibenzofuran, 90 mg/kg hexachlorodibenzofuran, 400 mg/kg heptachlorodibenzofuran, and 260 mg/kg octachlorodibenzofuran. From Monsanto Chemical Company.

Source: Compiled from Goldstein et al., 1977.

in the erythrocyte counts (7.75×10^6 vs 8.18×10^6), hemoglobin (15.3 g vs 16.7 g), and packed cell volume (46.4% vs 51.3%) in rats fed 30 mg technical pentachlorophenol per kilogram. There was no effect on the renal parameters measured. In rats fed technical pentachlorophenol the alkaline phosphatase increased (26.0 King-Armstrong units at 3 mg/kg and 25.7 King-Armstrong units at 30 mg/kg vs 20.5 King-Armstrong units for controls). Serum glutamic-pyruvic transaminase increased in the rats receiving high doses of technical pentachlorophenol (74.8 vs 61.0 Karmen units). Serum albumin decreased in the 30 mg/kg group (2.3 g per 100 ml)

TABLE D.6.28. COMPARISON OF THE TOXICITIES OF PURE AND TECHNICAL GRADE PENTACHLOROPHENOL IN MALE RATS IN A 90-DAY STUDY[a]

Parameter	Effect of pentachlorophenol at various levels[b]					
	Pure[c]			Technical grade[d]		
	3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Body weight	0	0	0	0	0	0
Food consumption	0	0	0	0	0	0
Hematology (five per group, day 83)						
Hemoglobin	0	0	0	0	0	<
Erythrocyte count	0	0	0	0	0	<
Packed cell volume	0	0	0	0	0	<
Total and differential leucocyte count	0	0	0	0	0	0
Urinalysis						
pH	ND	ND	0	ND	ND	0
Sugar	ND	ND	0	ND	ND	0
Albumin	ND	ND	0	ND	ND	0
Ketones	ND	ND	0	ND	ND	0
Occult blood	ND	ND	0	ND	ND	0
Clinical chemistry						
Blood urea nitrogen	0	0	>	0	0	0
Alkaline phosphatase	0	0	<	>	0	>
Serum glutamic-pyruvic transaminase	0	0	0	0	0	>
Albumin	0	0	0	0	<	<
Protein	0	0	0	0	0	0
Organ weight (absolute)						
Liver	0	0	>	0	>	>
Kidney	0	0	0	0	0	>
Organ weight (relative)						
Liver	0	>	>	>	>	>
Kidney	0	0	>	>	>	>

[a] Ten rats per group. Dietary levels were adjusted weekly to compensate for changes in body weights and rates of food consumption. Statistical analysis by Student's *t* test.

[b] 0 indicates no statistically significant change; > indicates a statistically significant increase; < indicates a statistically significant decrease; ND means not determined.

[c] Combination of 95% purified pentachlorophenol and 5% purified tetrachlorophenol containing <0.5 mg/kg dibenzodioxins or dibenzofurans.

[d] Production grade material containing 2500 mg/kg octachlorodibenzo-*p*-dioxin, 125 mg/kg heptachlorodibenzo-*p*-dioxin, 4 mg/kg hexachlorodibenzo-*p*-dioxin, 80 mg/kg octachlorodibenzofuran, 80 mg/kg heptachlorodibenzofuran, and 30 mg/kg hexachlorodibenzofuran.

Source: Compiled from Kociba et al., 1971. Results have also been reported in part by Schwetz, Gehring, and Kociba, 1973, and Johnson et al., 1973.

and the 10 mg/kg group (2.5 g per 100 ml) compared with the controls (2.7 g per 100 ml). There were no changes in total serum proteins. In rats fed the high dose of pure pentachlorophenol there was an increase in blood urea nitrogen (18 vs 15 mg per 100 ml) and a decrease in alkaline phosphatase (17.0 vs 21.4 King-Armstrong units). Relative liver and kidney weights increased at all dosage levels in the groups fed technical pentachlorophenol. In the groups receiving pure pentachlorophenol, relative liver weights increased in the two higher-dose groups and relative kidney weight increased in the high-dose group only. Pathologic changes were found only in the livers of rats fed the high dose of technical pentachlorophenol. Seven of ten rats showed minimal focal hepatocellular degenerative changes, and three of ten showed minimal focal hepatocellular necrosis. Both controls and treated groups had intestinal nematodiasis. It should be noted that these investigators used Student's *t* test in the statistical analysis of the data. As mentioned previously, more appropriate methods are available for making multiple comparisons of graded treatment levels with a common control group.

Kociba et al. (1973) fed male and female, seven-week-old, Sprague-Dawley rats pentachlorophenol with a low nonphenolic content for 90 days; the results are presented in Table D.6.29. The hematologic parameters were determined in the control and high-dose groups, and there were no differences. Similarly, the urinalysis and clinical chemistry variables were not altered. There were differences in liver and kidney weights in rats receiving higher doses. There were no treatment-related histopathologic changes. The no-effect level was reported as 3 mg/kg body weight.

Schwetz et al. (1976, 1978) fed weanling, male and female, Sprague-Dawley rats 0, 1, 3, 10, or 30 mg/kg pentachlorophenol with a low nonphenolic content for 24 months (females) or 22 months (males). The results are given in Table D.6.30. The changes observed in terminal samples were a decrease in body weight in high-dose females and an increase in serum glutamic-pyruvic transaminase activity in high-dose males and females. At one year, but not at the termination of the study, there was a small increase in urine specific gravity in high-dose females. The males were terminated two months earlier than the females because of death losses in male controls and the four male treatment groups. The study included microscopic examination of tissues. The incidence of changes was statistically analyzed by the Fisher exact probability test. An accumulation of pigment was observed in the liver and kidney of females fed 10 and 30 mg/kg. The brown, granular pigment in the liver was primarily found in the hepatocytes surrounding the central veins. In the kidney the pigment was in the proximal convoluted tubular epithelium. The pigment was not identified. It did not stain with any of the following techniques: oil red-O, nile blue sulfate, acid-fast, Gomori's stain for iron, McManus' periodic acid—Schiff, Hall's method for bilirubin, and Stein's method for bile pigments. The pigment was not observed grossly in the males but was found microscopically in the liver of one high-dose male. No other nontumorous pathologic changes related to ingestion of pentachlorophenol were observed.

TABLE D.6.29. TOXICITY OF PENTACHLOROPHENOL WITH A LOW NONPHENOLIC CONTENT TO RATS DURING A 90-DAY STUDY[a]

Parameter	Effect of pentachlorophenol at various levels[b]							
	Females[c]				Males[c]			
	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Body weight (final)	0	0	0	[illegible]	[illegible]	0	0	0
Food consumption	0	0	0	0	0	0	0	0
Hematology (five per group, day 83)								
Packed cell volume	ND	ND	ND	0	ND	ND	ND	0
Erythrocyte count	ND	ND	ND	0	ND	ND	ND	0
Hemoglobin	ND	ND	ND	0	ND	ND	ND	0
Total and differential leucocyte count	ND	ND	ND	0	ND	ND	ND	0
Urinalysis (five per group, day 83)								
Specific gravity	ND	ND	ND	0	ND	ND	ND	0
pH	ND	ND	ND	0	ND	ND	ND	0
Sugar	ND	ND	ND	0	ND	ND	ND	0
Protein	ND	ND	ND	0	ND	ND	ND	0
Ketones	ND	ND	ND	0	ND	ND	ND	0
Occult blood	ND	ND	ND	0	ND	ND	ND	0
Bilirubin	ND	ND	ND	0	ND	ND	ND	0
Clinical chemistry (five per group)								
Blood urea nitrogen	0	0	0	0	0	0	0	0
Alkaline phosphatase	0	0	0	0	0	0	0	0
Serum glutamic-pyruvic transaminase	0	0	0	0	0	0	0	0
Organ weight (absolute)								
Liver	0	0	0	0	0	0	[illegible]	[illegible]
Kidney	0	0	0	0	0	0	0	[illegible]
Organ weight (relative)								
Liver	0	0	0	[illegible]	0	0	[illegible]	[illegible]
Kidney	0	0	0	[illegible]	0	0	0	[illegible]

[a] Compound administered was a distilled form of Dowicide 7, considered to be representative of the improved product registered as Dowicide EC-7. Nonphenolic content was 1 mg/kg hexachlorodibenzo-p-dioxin, 6.5 mg/kg heptachlorodibenzo-p-dioxin, 15.0 mg/kg octachlorodibenzo-p-dioxin, 3.4 mg/kg hexadibenzofuran, and 1.8 mg/kg heptadibenzofuran.

[b] Data analyzed by analysis of variance and Dunnett's test. 0 indicates no statistically significant change; [illegible] indicates a significant increase; [illegible] indicates a significant decrease; ND means not determined.

[c] Ten males and ten females per group.

Source: Compiled from Kociba et al., 1973.

From the above studies it is apparent that the nonphenolic contaminants present in technical pentachlorophenol contribute to the biological activity of pentachlorophenol. The primary evidence of biological activity is enzyme induction. Dosage levels of 30 to 50 mg/kg body weight for 90 days to eight months are required to produce pathologic changes in the liver. This feeding pattern represents prolonged dosing at one-third to one-fifth the single-dose acute LD_{50}. The no-effect level in experimental animals appears to be 3 mg/kg. The level selected depends on the significance attached to changes in organ weights and to enzyme induction responses.

TABLE D.6.30. CHRONIC TOXICITY OF PENTACHLOROPHENOL WITH LOW NONPHENOLIC CONTENT TO MALE AND FEMALE RATS[a]

Parameter	Effect of pentachlorophenol at various levels[b]							
	Females[c]				Males[c]			
	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Body weight	0	0	0	<	0	0	0	0
Food consumption	0	0	0	0	0	0	0	0
Hematology								
Erythrocyte count	0	0	0	0	ND	ND	ND	0
Hemoglobin	0	0	0	0	ND	ND	ND	0
Total and differential leucocyte count	0	0	0	0	ND	ND	ND	0
Clinical chemistry								
Blood urea nitrogen	0	0	0	0	0	0	0	0
Alkaline phosphatase	0	0	0	0	0	0	0	0
Serum glutamic-pyruvic transaminase	0	0	0	>	0	0	0	>
Urinalysis								
pH	0	0	0	0	ND	ND	ND	0
Specific gravity	0	0	0	0	ND	ND	ND	0
Sugar	0	0	0	0	ND	ND	ND	0
Albumin	0	0	0	0	ND	ND	ND	0
Ketones	0	0	0	0	ND	ND	ND	0
Bilirubin	0	0	0	0	ND	ND	ND	0
Occult blood	0	0	0	0	ND	ND	ND	0
Organ weight (absolute)								
Liver	0	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0	0
Organ weight (relative)								
Liver	0	0	0	0	0	0	0	0
Kidney	0	0	0	0	0	0	0	0

[a] Composition of pentachlorophenol same as that given in Table D.6.29. In addition, the pentachlorophenol contained 400 mg/kg hexachlorobenzene. Dietary levels were adjusted monthly.

[b] Data analyzed by Dunnett's test. 0 indicates no significant change from the control; > indicates a significant increase; < indicates a significant decrease; ND means not determined.

[c] 25 males and 25 females per group.

Source: Compiled from Schwetz et al., 1976, 1978.

D.6.2.2.4.2 In humans — Bevenue et al. (1967) surveyed the pentachlorophenol content of urine from a cross-section of Hawaiians (Table D.6.31). As one might expect, substantial pentachlorophenol was found in the urine of individuals occupationally exposed to the compound; amounts ranged from 3 to 35,700 μg/liter. A somewhat surprising result, however, was the detection of substantial pentachlorophenol levels in the urine of the general population. Levels of pentachlorophenol from 0 to 1840 μg/liter were found in the urine of persons not occupationally exposed.

The presence of pentachlorophenol in the urine of the general population was confirmed by Cranmer and Freal (1970), who reported levels of pentachlorophenol ranging from 2 to 11 μg/liter (Table D.6.32). The source of this exposure is unclear. Casarett et al. (1969) suggested that respiratory tract absorption is a reasonable explanation for the nearly ubiquitous occurrence of pentachlorophenol in human urine. The presence of pentachlorophenol in the environment, in food supplies, and in containers treated with preservatives may contribute to this low-level contamination in humans. Contamination is particularly likely in areas where pentachlorophenol is used widely as a wood preservative and/or herbicide. In Hawaii, pentachlorophenol is used on nearly all wood products to prevent termite infestation and has been sprayed on pineapple fields as a herbicide.

The question of possible long-term chronic effects resulting from low-dose pentachlorophenol exposure remains open. Evidence exists that low-dose pentachlorophenol exposure does not result in chronic effects. Arsenault (1976), in a review of epidemiologic studies since 1967 at the University of Hawaii Pacific Biomedical Research Center at Manoa, stated that long-term chronic effects of low-dose pentachlorophenol exposure appear to be absent:

> In a prospective study of an occupationally exposed population annual physical examinations, blood chemistry and urinalysis testing were undertaken on all control and occupationally exposed adults. There were 21 workers having a mean of 3196 total days of exposure to PCP in an occupation of treating wood (pressure treatment). Although PCP was used by 70 other workers, such as pest control operators, the wood treaters had significantly higher levels of PCP than all other participants in the study. The mean blood serum level in the 21 workers was 1.05 ppm whereas the control was about 0.1 ppm. While vertigo and insomnia occurred more frequently among workers with high serum PCP levels, in tests of workers for neurological and psychological effects, there were no effects noted for PCP residue levels. In fact, PCP was the only pesticide of the four studied (PCP, Dieldrin, DDE, and DDT) that showed an insignificant contribution in a regression study.

However, Klemmer (1972) noted that the liver enzymes serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and lactic dehydrogenase were at their highest mean levels in persons occupationally

TABLE D.6.31. CONCENTRATIONS OF PENTACHLOROPHENOL IN URINE OF HAWAIIANS

Group	Firm No.	Number of people	Pentachlorophenol in urine (mg/liter) Low	High	Mean
Occupationally exposed pest control operators	1	19	0.003	0.23	0.064
	2	15	0.010	35.7	1.9
	3	12	0.040	16.6	2.2
	4	12	0.040	1.2	0.29
	5	11	0.034	6.4	1.5
	6	5	0.017	0.28	0.11
	7	5	0.052	7.2	1.7
	8	5	0.029	0.19	0.098
	9	4	0.016	3.4	0.45
	10	4	0.010	2.8	0.70
	11	3	0.034	4.2	0.82
	12	3	0.78	27.5	13.0
	13	3	0.11	1.4	0.42
	14	3	0.096	0.20	0.14
	15	3	0.021	0.31	0.13
	16	3	0.012	0.10	0.064
	17	2	0.60	6.2	3.5
	18	2	2.4	15.4	7.2
	19	2	0.61	11.7	6.2
	20	2	0.26	6.8	3.5
	21	2	0.031	0.44	0.23
	22	2	0.070	0.075	0.073
	23	1	2.6	12.0	6.2
	24	1	2.0	3.8	3.0
	25	1			1.6
	26	1			1.0
	27	1			0.53
	28	1			0.26
	29	1			0.12
	30	1			0.028
Total		130	0.03	35.7	1.8
Nonoccupationally exposed persons					
Households		32	0.007	0.065	0.031
Mosquito control		23	0.005	0.44	0.033
Office workers		18	0.010	0.043	0.021
Orchid growers		12	[a]	0.11	0.026
Rodent control		10	0.003	0.034	0.014
Hawaii Housing Authority		10	0.008	1.8	0.19
Hawaii Department of Agriculture		5	0.036	0.16	0.078
City and county of Honolulu		4	0.021	0.37	0.17
Division of Forestry		3	0.022	0.15	0.077
Total		117		1.8	0.040

[a] Not detected.

Source: Adapted from Bevenue et al., 1967, Table 1, pp. 322-323. Reprinted by permission of the publisher.

TABLE D.6.32. CONCENTRATION RANGE OF PENTACHLOROPHENOL IN HUMAN URINE

Persons sampled	Pentachlorophenol in urine (μg/liter)	
	Range of four replicates	Mean
General population	2.2-2.3	2.2
	2.7-2.9	2.8
	2.9-3.0	2.9
	5.0-5.3	5.1
	5.2-5.5	5.3
	10.6-11.2	10.8
Carpenter	22.2-25.5	24.1
Boat builder	55.0-60.1	57.3
Sprayer	131-136	133
	259-270	265

Source: Adapted from Cranmer and Freal, 1970, Table III, p. 125.

exposed to pentachlorophenol. The clinical significance of this finding is not known.

Recent data reported by Begley et al. (1977) indicate that chronic exposure to pentachlorophenol may have reversible effects on kidney function. Blood and urine samples from 18 male volunteers employed by a firm which treated lumber and other wood products with 5% pentachlorophenol were used in determining levels and clearance values for creatinine, phosphorus, and pentachlorophenol before, at intervals during, and following a 20-day vacation. Significant differences for blood and urinary phosphorus levels and creatinine clearance were found before, during, and after vacation. Begley et al. (1977) concluded that pentachlorophenol exposure results in decreased creatinine clearance and phosphorus reabsorption in the kidney and that the effects are reversible. Spontaneous improvement was noted during the vacation interval.

Workers chronically exposed to pentachlorophenol demonstrated significantly elevated levels of total bilirubin and creatine phosphokinase, although Takahashi et al. (1975) reported that the levels were within normal limits. This study also indicated that workers chronically exposed to pentachlorophenol showed a significantly higher prevalence of gamma-mobility C-reactive protein in the sera. The clinical significance of these elevated levels in persons exposed to pentachlorophenol is not known. Takahashi et al. (1975) noted, however, that C-reactive protein levels are often elevated in acute states of various inflammatory disorders or tissue damage. It was therefore inferred that the elevated levels of C-reactive protein in individuals exposed to pentachlorophenol indicate inflammation or tissue injury.

The greatest risk of pentachlorophenol exposure obviously exists among those engaged in the manufacture and use of the compound. The general population may be exposed to pentachlorophenol via atmospheric contamination, the presence of pentachlorophenol in water and food, and possible cutaneous contamination from pentachlorophenol-treated items. No data are available on ambient levels of pentachlorophenol in the atmosphere; therefore, pentachlorophenol uptake via the respiratory route remains speculative.

Arsenault (1976) reported pentachlorophenol levels in air of companies using pentachlorophenol as a wood preservative (Table D.6.33). The average worker exposure was defined as the atmospheric concentration found in the area where a worker spends the majority of his time and the maximum worker exposure as that found in the maximum exposure area near the pentachlorophenol source. Normal physiologic data indicate that a person performing light work breathes approximately 4.8 m^3 of air during 8 hr. The calculated total pentachlorophenol inhalation exposure would range from 0.03 to 1.42 mg. For an individual doing moderately heavy work for 8 hr, the approximate total pulmonary ventilation would be 14.4 m^3 with pentachlorophenol inhalation exposures ranging from 0.08 to 4.28 mg. Humans normally excrete approximately 1.4 liters of urine per day; thus, the total pentachlorophenol content in urine, as calculated from the data in Table D.6.33, would range from 1.4 to 4.0 mg per person per day. Based on these calculations, exposure and excretion are approximately balanced. Undoubtedly, there is also some dermal exposure, and it is unlikely that there is 100% retention of inhaled pentachlorophenol.

TABLE D.6.33. PENTACHLOROPHENOL CONCENTRATIONS IN AIR FROM PLANTS AND URINE OF MILL WORKERS IN OREGON

Type of operation	Pentachlorophenol in air (mg/m^3)				Pentachlorophenol in urine (mg/liter)	
	Average worker exposure		Maximum worker exposure			
	Mean	Range	Mean	Range	Mean	Range
Dip	0.019	0.003-0.063	0.019	0.006-0.063	2.83	0.12-9.68
Spray	0.006	0.003-0.012	0.026	0.004-0.069	0.98	0.13-2.58
Pressure	0.014	0.004-0.028	0.297	0.043-1.000	1.24	0.17-5.57

Source: Adapted from Arsenault, 1976, Table 1, p. 8. Reprinted by permission of the publisher.

A serious concern is the possible bioaccumulation of pentachlorophenol in adipose tissue. The chemical structure of the compound suggests the possibility of accumulation in fat, and recent data have indicated that this may occur. Apparently, pentachlorophenol accumulates in fish (Section D.5.2.2.3). Pentachlorophenol has also been detected in human adipose tissue. Autopsy results from an infant fatally poisoned by pentachlorophenol (Table D.6.6) showed a higher level of pentachlorophenol in the fat than in any other tissue examined (Armstrong et al., 1969). A survey by Shafik (1973) indicated the presence of low levels of pentachlorophenol in adipose tissue samples from the general population. Levels of 12 to 45 μg/kg were found in individuals not occupationally exposed to the substance (Table D.6.34). Despite the detection of pentachlorophenol in fat tissue, no evidence presently indicates a significant accumulation of pentachlorophenol in mammalian fat tissue. More data are needed to fully resolve this question.

TABLE D.6.34. PENTACHLOROPHENOL LEVELS IN ADIPOSE TISSUE OF NONOCCUPATIONALLY EXPOSED PERSONS (μg/kg)

Sample No.	Pentachlorophenol in adipose tissue
1	19
2	17
3	52
4	45
5	23
6	25
7	21
8	35
9	17
10	20
11	22
12	14
13	35
14	52
15	24
16	12
17	12
18	29

Source: Adapted from Shafik, 1973, Table 1, p. 62. Reprinted by permission of the publisher.

D.6.2.2.5 Exposure, Safety, and Pentachlorophenol Intoxication — Fatalities due to pentachlorophenol poisoning are about equally divided between people poisoned by contact with pentachlorophenol and those poisoned with sodium pentachlorophenate. In almost all cases, fatal and nonfatal, misuse of the compound was paramount.

A number of fatalities have occurred when sodium pentachlorophenate was used as a molluscicide or as a herbicide. According to Blair (1961) and Gordon (1956), the workers involved in the spraying operations were inadequately protected from cutaneous contact with the spray.

Fatal poisonings by pentachlorophenol have also occurred in timber dipping operations. In the cases reported by Truhaut, Boussemart, and L'Épée (1952) and by Bergner, Constantinidis, and Martin (1965), the workers were either dipping wood in preservative solutions or handling newly treated lumber without gloves. Because pentachlorophenol can pass through the skin without overt damage, considerable risk is involved in handling the compound without the protection of gloves.

In the pentachlorophenol manufacturing industry, poisoning has occurred in some cases because toxic levels of dust, aerosol, or vapor build up in enclosed areas due to inadequate ventilation. Thus, poor hygiene lies at the root of many of the poisonings. A study by Arsenault (1976) illustrated the effect of good industrial hygiene in handling this very hazardous compound. A review of the medical records of the 28 wood preserving plants operated by the Koppers Company, which covered approximately 1670 employees over a ten-year span, revealed only 26 cases of pentachlorophenol-related health problems (Table D.6.35).

Nonoccupationally exposed persons have also been involved in pentachlorophenol poisoning incidents. Two fatalities in a St. Louis nursery apparently resulted from the improper use of a formulation containing sodium pentachlorophenate in the laundering of diapers and infants' bed linens (Robson et al., 1969). A California housewife suffered from pentachlorophenol intoxication following the improper use of pentachlorophenol to prevent graying of the paneling inside her new home (Anonymous, 1970).

A series of papers by Duggan and Lipscomb (1969), Duggan and Corneliussen (1972), and Manske and Corneliussen (1974) provide estimates of the average dietary intake of various pesticide chemicals in the United States. Pentachlorophenol was one of the chemicals studied, and the composite data are shown in Tables D.6.36 and D.6.37. The major conclusion that can be drawn from these data is that pentachlorophenol is occasionally present in food products. The levels found, although small, represent a source of pentachlorophenol contamination for humans. In areas where pentachlorophenol is widely used, food contamination may be substantially higher than the values recorded.

Possible exposure may also occur via drinking water. Buhler, Rasmusson, and Nakaue (1973) monitored pentachlorophenol levels in sewage influent collected from three Oregon cities, the composite effluent from the same sewage treatment plants, the Willamette River just upstream from Corvallis, Oregon, and the drinking water processed at the Taylor Water Treatment Plant in Corvallis (Tables D.6.38 and D.6.39 and Figure D.6.19). Pentachlorphenol levels in the 24-hr composite samples of sewage influent collected from the three Oregon cities ranged from 1 to 5 μg/liter. Composite effluent values ranged from 1 to 4 μg/liter,

TABLE D.6.35. CASES OF PENTACHLOROPHENOL-RELATED HEALTH PROBLEMS REPORTED BY EMPLOYEES OF WOOD PRESERVING PLANTS OPERATED BY THE KOPPERS COMPANY OVER A TEN-YEAR PERIOD

Health problem	Number of cases reported
Chemical conjunctivitis	12
Skin burns	9
Allergy	1
Dermatitis (arms and hands)	2
Miscellaneous[a]	2
Total	26

[a] One case of gastritis from inhalation of pentachlorophenol and one case of folliculitis on legs from continually wearing pentachlorophenol-contaminated trousers.

Source: Adapted from Arsenault, 1976, p. 9. Reprinted by permission of the publisher.

TABLE D.6.36. AVERAGE INCIDENCE AND DAILY INTAKE OF PENTACHLOROPHENOL IN FOOD IN THE UNITED STATES

Year	Number of composite samples	Samples containing pentachlorophenol (%)	Average daily intake (mg per person)
1965	216	1.4	<0.001
1966	332	3.3	0.006
1967	360	2.2	0.001
1968	360	1.9	0.001
1969	360	2.8	0.002

Source: Compiled from Duggan and Corneliussen, 1972.

TABLE D.6.37. AVERAGE DAILY INTAKE OF PENTACHLOROPHENOL FROM EIGHT FOOD CLASSES IN THE UNITED STATES (mg per person)

Food	Pentachlorophenol intake			
	6/66-4/67	6/67-4/68	6/68-4/69	6/70-4/71
Beverages	0.001			
Dairy products	<0.001	0.001	0.001	<0.001
Grains and cereals	<0.001	0.001	0.001	
Meat, fish, and poultry	<0.001	<0.001	0.001	
Oils, fats, and shortening	<0.001		0.001	
Potatoes		<0.001		
Garden fruits			<0.001	
Sugars and adjuncts			<0.001	0.011[a]

[a] In micrograms per gram of food.

Source: Compiled from Duggan and Lipscomb, 1969; Duggan and Corneliussen, 1972; Manske and Corneliussen, 1974.

reflecting a 4% to 28% removal of pentachlorophenol. Pentachlorophenol levels in the Willamette River water were from 0.1 to 0.7 µg/liter. Conventional processing of the river water removed about 60% of the pentachlorophenol, leaving approximate levels of 0.06 µg/liter in the finished drinking water. Therefore, persons in these three cities apparently are exposed to constant low levels of pentachlorophenol in drinking water. The pentachlorophenol originally found in sewage treatment plant influent probably resulted from discharge by an industry using the substance as a preservative and for slime control, as well as from the rather wide use of pentachlorophenol in a variety of fungicidal or germicidal applications.

Municipal chlorination of drinking water can produce chlorinated phenols in the microgram per liter range. Highly chlorinated phenols are formed by this chlorination procedure; chlorine levels of 10 ppm can chlorinate 1 ppm phenol, generating 0.2 ppb of pentachlorophenol (Arsenault, 1976). Phenol may be present as a result of degradation of plant material or by discharge into natural water bodies. Thus, municipal chlorination may produce low-level pentachlorophenol contamination in treated water supplies.

High levels of pentachlorophenol have been found in river water where pentachlorophenol is widely dispersed in the environment. Goto (1971)

TABLE D.6.38. LEVELS OF PENTACHLOROPHENOL IN 24-hr COMPOSITE INFLUENT AND EFFLUENT SAMPLES AND TOTAL PENTACHLOROPHENOL OUTPUT FROM SEWAGE TREATMENT PLANTS IN OREGON

City	Population	Pentachlorophenol level (μg/liter)		Pentachlorophenol removed (%)	Total pentachlorophenol output	
		Influent	Effluent		(g/day)	(g per 10,000 people per day)
Corvallis	38,000	1.4	1.0	28.6	20	5.5
Eugene	60,000	4.1	3.3	19.5	144	26.0
Salem	80,000	4.6	4.4	4.4	267	33.6

Source: Adapted from Buhler, Rasmusson, and Nakaue, 1973, Table IV, p. 932. Reprinted by permission of the publisher.

TABLE D.6.39. CONCENTRATION AND REMOVAL OF PENTACHLOROPHENOL AT VARIOUS STAGES OF THE WATER TREATMENT PROCESS AT THE TAYLOR PLANT IN CORVALLIS, OREGON

Stage sampled	Time sampled[a]	Pentachlorophenol concentration (μg/liter)	Pentachlorophenol removed (%)
Raw Willamette River water (influent)	8:00 AM	0.17	
At flocculation step	8:27 AM	0.06	65
After sedimentation	10:05 AM	0.04	76
After filtration (final product)	10:15 AM	0.06	65

[a] Sampling times were calculated on the basis of known volumes, delay times, and a water flow of 25,350 liters/min pumped into the treatment plant.

Source: Adapted from Buhler, Rasmusson, and Nakaue, 1973, Table V, p. 933. Reprinted by permission of the publisher.

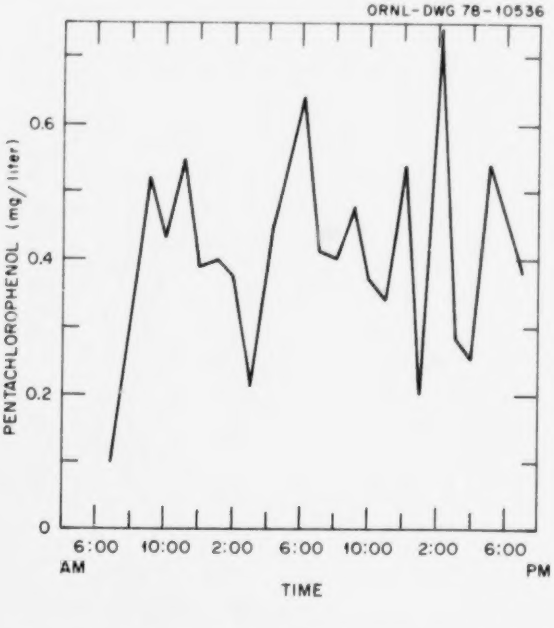

Figure D.6.19. Hourly concentrations of pentachlorophenol in the Willamette River on September 30, 1969. Source: Adapted from Buhler, Rasmusson, and Nakaue, 1973, Figure 2, p. 932. Reprinted by permission of the publisher.

reported levels of pentachlorophenol as high as 10 μg/liter in river water in Japan (Figure D.6.20). In Japan pentachlorophenol is widely used as a herbicide during rice transplantation. High pentachlorophenol levels in water from the portion of a river downstream from a pulp mill were reported by Rudling (1970).

One source of human contamination by pentachlorophenol which has often been overlooked is the possible presence of the substance as a degradation product of other compounds in the body. Preliminary evidence from Yang, Coulston, and Goldberg (1975) suggested the possible formation of pentachlorophenol in the rhesus monkey following administration of hexachlorobenzene. Lui and Sweeney (1975) and Mehendale, Fields, and Matthews (1975) isolated pentachlorophenol from the urine of rats dosed with hexachlorobenzene; pentachlorophenol was not detected in the feces. Mehendale, Fields, and Matthews (1975) also showed that microsomal preparations from rat liver were able to produce one or more chlorophenols, including pentachlorophenol. Karapally, Saha, and Lee (1973) used gas chromatography to tentatively identify pentachlorophenol in the urine of rabbits which had been administered a dose of ^{14}C-labeled lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane). Engst, Macholz, and Kujawa (1976) gave rats 8 mg hexachlorobenzene per kilogram for 19 days. Analysis of the tissues revealed hexachlorobenzene and pentachlorophenol as the main metabolite. Small amounts of 2,3,4,6- and 2,3,5,6-tetrachlorophenol, 2,4,6-trichlorophenol, and pentachlorobenzene were also detected. Engst et al. (1976) also demonstrated that pentachlorophenol, tetrachlorophenols, and trichlorophenols are metabolites of the insecticide lindane in the rat. These studies offer an alternative explanation for the nearly ubiquitous presence of pentachlorophenol in humans and question the tacit assumption that pentachlorophenol in human urine, serum, and tissues results from exposure to pentachlorophenol. Additional investigations to determine the

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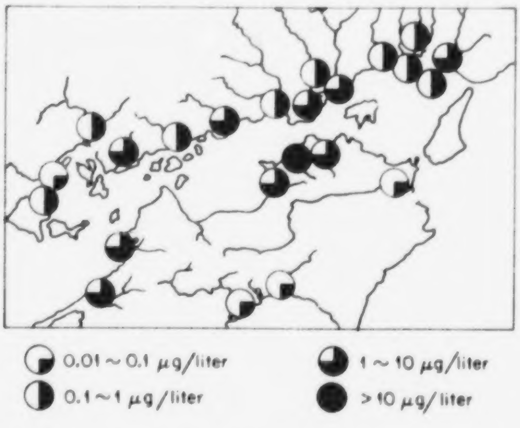

Figure D.6.20. Pentachlorophenol residues in river water in southwestern Japan during July 1969. Source: Adapted from Goto, 1971, Figure 2, p. 109.

extent of pentachlorophenol synthesis from other compounds present in human tissues are urgently needed.

D.6.2.3 Carcinogenicity

Innes et al. (1969) found no significant indication of tumorigenicity following oral administration of pentachlorophenol to mice. Schwetz et al. (1978) fed rats pentachlorophenol in a lifetime study. No treatment-related increase in tumors was found (Table D.6.40). Boutwell and Bosch (1959) reported that repeated applications of phenol and some substituted phenols can promote the appearance of skin tumors in mice following a single initiating dose of dimethylbenzanthracene. Tumors also developed in mice (not exposed to dimethylbenzanthracene) treated with phenol alone for long periods. When pentachlorophenol was tested in a similar system, tumorigenicity and tumor-promoting activity were absent. Thus, no evidence exists that pentachlorophenol possesses carcinogenic characteristics.

D.6.2.4 Teratogenicity

Pentachlorophenol does not appear to be teratogenic in the rat (Schwetz, Keeler, and Gehring, 1974; Larsen et al., 1975). Although some degree of malformation has been observed, the number was generally minimal and could have been due to the toxic effects of the compound on the maternal rat or, if placental transfer occurs, a direct fetotoxic effect on the developing embryo. Following the administration of pentachlorophenol to rats, signs of embryotoxicity and fetotoxicity, such as resorptions, subcutaneous edema, dilated ureters, and anomalies of the skull, ribs, vertebrae, and sternebrae, were observed at an incidence which increased with increasing doses. Larsen et al. (1975) indicated that placental transfer of pentachlorophenol is minimal and that the fetotoxic effects likely resulted from a direct effect on the maternal rat. The most prominent effect of pentachlorophenol on the mammalian system is hyperpyrexia, and this effect alone may be the cause of the fetotoxicity. The no-effect level of pentachlorophenol in the rat has been reported by Schwetz, Gehring, and Kociba (1973) to be 5 mg/kg body weight per day. Hinkle (1973) administered 1.25 to 20 mg/kg on day 5 to day 10 of gestation in Golden Syrian hamsters. Fetal deaths and/or resorptions were observed in three of six tests groups (unspecified).

D.6.2.5 Mutagenicity

Pentachlorophenol did not exhibit mutagenic properties when tested in a microbiological system (Anderson, Leighty, and Takahashi, 1972), in a mammalian test system (Buselmaier, Röhrborn, and Propping, 1973), or in the fruit fly, *Drosophila melanogaster* (Vogel and Chandler, 1974).

TABLE D.6.40. INCIDENCE OF PRIMARY TUMORS (BASED ON HISTOPATHOLOGICAL DIAGNOSIS) IN RATS FED PENTACHLOROPHENOL FOR 22 MONTHS (MALES) AND 24 MONTHS (FEMALES)

	Males					Females				
	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg
Number of rats examined	27	26	27	27	27	27	27	27	27	27
Number of rats with tumors	11	13	13	12	11	27	26	25	25	25
Number of tumors	17	14	17	15	16	62	67	42	63	63
Number of tumors/rats with tumors	1.6	1.1	1.3	1.3	1.4	2.3	2.6	1.7	2.5	2.5
Number of morphologic malignant tumors	1	3	2	1	0	2	7	2	3	2

Source: Adapted from Schwetz et al., 1978, Table 5, p. 307. Reprinted by permission of the publisher.

SECTION D.6

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D.7 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

D.7.1 TRENDS IN PRODUCTION AND USE

Pentachlorophenol and sodium pentachlorophenate have been used extensively as broad-spectrum biocides. In 1972, annual production in the United States alone exceeded 50,000,000 lb (23,000,000 kg) (Anonymous, 1972). Growth was projected at about 4% per annum after 1972. Estimated production of pentachlorophenol and sodium pentachlorophenate in 1976 was 56,000,000 lb (25,400,000 kg). About 90% to 95% of this amount is used in the wood preservation industry. The remaining 5% to 10% has a large number of applications in industry and agriculture.

Pentachlorophenol (or sodium pentachlorophenate) has been used as a wood preservative to control mold and termite infestations, as a weedicide or preharvest desiccant, as an algicide, as a molluscicide for the eradication of the snail which serves as intermediate host for the human schistosomes, and in food processing plants and pulp mills for the control of slime and mold. Pentachlorophenol and sodium pentachlorophenate also have been used as fungicides and/or bactericides in the processing of cellulosic products, starches, adhesives, paints, leathers, oils, rubber, textiles, and wooden crates used for packaging raw agricultural products. Obviously, a product with such wide and varied uses has a serious potential for environmental contamination (Bevenue and Beckman, 1967).

Pentachlorophenol has been manufactured by five chemical companies in five states. The locations and production capacities of these companies are given in Table D.7.1. Recently, Sonford Chemical Company and Monsanto Industrial Chemicals Company terminated production or indicated an intent to stop production. Pentachlorophenol is produced in widely separated states. Unlike some agricultural pesticides which are restricted to an area of intense application, pentachlorophenol is widely used as an industrial preservative; thus, the logistics of distribution require that it be produced at scattered locations.

The potential for pollution of the environment by pentachlorophenol obviously exists at point sources of pollution such as industries that manufacture or use the compound in large amounts. The widespread use of the compound in the environment as a wood preservative and as a general biocide also allows for environmental contamination through leaching from preservative-treated wood; direct contamination of soil, air, and water when the compound is used as a weedicide; and release of the compound from preservative-treated items either during use or after disposal.

D.7.2 SOURCES OF POLLUTION

D.7.2.1 Distribution in Air

Information on atmospheric levels of pentachlorophenol is scanty. Casarett et al. (1969) conducted a survey of blood and urine pentachlorophenol concentrations among workers employed by a factory involved in wood

TABLE D.7.1. PRODUCERS OF PENTACHLOROPHENOL IN THE UNITED STATES

Company and location	Annual pentachlorophenol production capacity		Estimated pentachlorophenol production, 1974	
	(lb x 10^6)	(kg x 10^6)	(lb x 10^6)	(kg x 10^6)
Dow Chemical Company, Midland, Michigan	18	8.1	5	2.2
Monsanto Industrial Chemicals Company, Sauget, Illinois	26	11.8	10	4.4
Reichhold Chemicals, Inc., Tacoma, Washington	16	7.2	10	4.4
Sonford Chemical Company, Houston, Texas	18	8.1	5.2	2.3
Vulcan Materials Company, Wichita, Kansas	19	8.6	18.7	8.4
Total	97	43.8	48.9	21.7

Source: Adapted from Ifeadi, 1975, Table 10, p. 45.

preservation. This survey indicated substantial pentachlorophenol content in blood and urine compared with that of nonoccupationally exposed persons; qualitatively significant concentrations of pentachlorophenol were detected in the plant atmosphere. Because of widespread pentachlorophenol distribution in the factory yard, the workers were believed, in part, to have received respiratory intoxication. Bevenue et al. (1967) found substantial pentachlorophenol levels in the urine of nonoccupationally exposed persons in Hawaii. Although the source was uncertain, Casarett et al. (1969) suggested that respiratory tract absorption was a reasonable explanation for the nearly ubiquitous occurrence of pentachlorophenol in the urine of Hawaiians. Pentachlorophenol is used widely in Hawaii to protect wooden structures against termite infestation, and therefore low-level pentachlorophenol contamination of the atmosphere is a likely possibility. No direct data on ambient pentachlorophenol levels in the atmosphere are available. Circumstantial evidence was provided by Bevenue, Ogata, and Hylin (1972), who found levels of pentachlorophenol in rainwater collected in Hawaii ranging from 2 to 284 ng/liter (Table D.7.2). The pentachlorophenol in the rainwater likely came from washout of pentachlorophenol present in the atmosphere either as a vapor or as an occlusion on dust particles. These authors also found 14 ng pentachlorophenol per liter

TABLE D.7.2. PENTACHLOROPHENOL IN RAINWATER, OAHU, HAWAII, 1971-1972

Sampling site	Period	Pentachlorophenol content (ng/liter)
Kailua	1-5-72 to 1-23-72	20
Kaneohe	9-30-71 to 11-17-71	8
	1-4-72 to 1-25-72	11
	1-28-72 to 2-23-72	16
	2-28-72 to 3-4-72	15
	3-4-72 to 4-27-72	10
Waipahu	1-6-72 to 3-4-72	14
Honolulu		
Nuuanu-Vinyard Avenues	1-6-72 to 3-4-72	16
Nuuanu-School Streets	9-28-71 to 12-27-71	77
Nuuanu-School Streets	1-1-72 to 2-23-72	45
Nuuanu-School Streets	2-25-72 to 5-15-72	2
Pawaa Lane	1-1-72 to 2-20-72	55
Pawaa Lane	2-27-72 to 5-11-72	50
Manoa	9-30-71 to 11-15-71	284
Manoa	1-3-72 to 1-24-72	270

Source: Adapted from Bevenue, Ogata, and Hylin, 1972, Table 1, p. 239. Reprinted by permission of the publisher.

in snow samples from Mauna Kea Summit and 10 ng/liter in water from Lake Waiau, which is fed almost exclusively by the summit snows.

Airborne pentachlorophenol may be toxic to conifer seedlings (Ferguson, 1959). The damage noted to plants grown in flats treated with pentachlorophenol apparently resulted from volatilization of the compound. Pentachlorophenol vapor is also toxic to man. Treatment of the interior redwood paneling of a home resulted in intoxication of the inhabitant, presumably by volatilization of pentachlorophenol from the paneling (Anonymous, 1970). Thus, potential exists for volatilization of pentachlorophenol from preservative-treated objects, thereby increasing the pentachlorophenol levels in the air. However, the low vapor pressure of pentachlorophenol (0.00011 mm Hg at 20°C) suggests that volatilization is a slow process. Furthermore, the use of oils and other hydrocarbon solvents further reduces volatilization. Intuitively, if pentachlorophenol vaporized very rapidly from treated wood, where 90% of pentachlorophenol is used, then its use as a wood preservative would be very limited (Environmental Health Advisory Committee, 1978; Thompson, McGinnis, and Ingram, 1978).

Pentachlorophenol may volatilize from soil or water. Hilton, Yuen, and Nomura (1970) attributed a pronounced decrease in the pentachlorophenol content of an aerated solution to volatilization. Volatilization from water may be a significant source of atmospheric pentachlorophenol, especially in areas where it is applied as a herbicide in paddy fields or used as a molluscicide. Adsorption to soil is discussed in Section D.2.5.3, but no data are available for an evaluation of pentachlorophenol losses from soil by volatilization. Research in this area is warranted.

D.7.2.1.1 Point Sources — The potential for emission of large quantities of pentachlorophenol into the atmosphere exists in factories where pentachlorophenol is manufactured or used widely. Factories using high pressures and temperatures for wood preservation possess the potential for widespread distribution of pentachlorophenol into the atmosphere. No information on pollution control of atmospheric emissions by the wood preserving industry was found. A survey was made by Ifeadi (1975) in an attempt to develop background information on air pollution from pesticide manufacturing factories. This information, compiled from published data and "scanty responses from the pesticide manufacturers," was used to determine the efficiency of pollution control methods and the rates of emission for various compounds during the manufacture of pentachlorophenol (Table D.7.3). No site visits or samplings were made. Following treatment of gaseous emissions with a scrubber, the emission of pentachlorophenol amounted to 4.32 lb/ton (2.16 kg/metric ton) of pentachlorophenol manufactured. Thus, based on a nationwide production of 23,000,000 kg per year, about 50,000 kg is emitted. Because these values were not verified by plant visits and samplings, these figures may be in error. Ifeadi (1975) noted that the control of particulate emission by using bag filters is inefficient and needs augmentation. Controlled emission at this stage of manufacture is reported as 0.55 kg/metric ton. Based on an annual production for a single plant of 5,000,000 kg pentachlorophenol, approximately 2750 kg would be emitted in the particulate form. The effect of

TABLE D.7.3. AIR CONTAMINANT CONTROL METHODS USED IN PENTACHLOROPHENOL MANUFACTURE

Control method	Efficiency (%)	Controlled emission	
		(lb/ton)	(kg/metric ton)
Wet-packed and venturi scrubber (with water)	99-100	4.32[a]	2.16
Dust collector (bag filters)			
At ingot casting	99	0.1[b]	0.05
At shotting operation	95	1[b]	0.5

[a] Controlled emission reported by Monsanto Industrial Chemicals Company based on 1974 production.

[b] Controlled emission reported by Reichhold Chemicals, Inc., based on 1974 production.

Source: Adapted from Ifeadi, 1975, Table 12, p. 52.

this quantity of a compound which is potentially as toxic as pentachlorophenol on the immediate environment surrounding the factory is not known. Furthermore, because figures supplied to Ifeadi (1975) did not include efficiency data on air contaminant control from three of the five chemical firms involved in manufacturing pentachlorophenol, these levels must be considered a lower limit. Firms not reporting efficiency data may be releasing large quantities to the atmosphere. Because of the known toxic effects of airborne pentachlorophenol as well as the ability of pentachlorophenol to bioaccumulate in aquatic organisms, a detailed evaluation of the amounts discharged to the atmosphere is needed. The effects of nonlethal amounts of pentachlorophenol in the atmosphere on man and the environment have not been adequately assessed.

D.7.2.1.2 Nonpoint Sources — Because of the total lack of atmospheric monitoring information, the prime sources of nonpoint pollution are only speculative. Likely sources of atmospheric pollution with pentachlorophenol include volatilization from water, soil, impervious surfaces, and pentachlorophenol-treated objects.

D.7.2.2 Distribution in Aquatic Environments

D.7.2.2.1 Point Sources — Point sources for pentachlorophenol pollution of water are of two basic types: (1) factories which manufacture pentachlorophenol or use it in wood treatment or slime control and (2) municipal discharge from sewage treatment plants. Substantial levels of pentachlorophenol have been found in effluents from some municipal sewage treatment plants (Buhler, Rasmusson, and Nakaue, 1973).

Large amounts of pentachlorophenol discharged into receiving waterways when spills or industrial accidents occur have a lethal effect on aquatic organisms. In many cases, storm runoff carries pentachlorophenol to receiving waterways from industrial sites where large quantities of pentachlorophenol or pentachlorophenol-treated lumber are stored. Following a heavy rain, Bevenue et al. (1972) detected 1.14 μg/liter pentachlorophenol in a ditch which drained the grounds of a wood treatment plant. A large pile of pentachlorophenol-treated lumber drenched by the rain was believed to be the source of pentachlorophenol detected in the drainage ditch. Thompson and Dust (1971) analyzed raw wastewater from five wood treatment plants using pentachlorophenol and creosote. The pentachlorophenol content of the untreated wastewaters from these plants ranged from 25 to 150 mg/liter. Sewage treatment reduced these levels substantially (Section D.7.4). Fountaine, Joshipura, and Keliher (1976) reported levels of 0.1 to 10 ppm pentachlorophenol in water taken from a stream at various distances from the outflow of a wood preserving company.

Pierce et al. (1977) determined pentachlorophenol residues in water, fish, leaf litter, and lake sediment for 17 months after pentachlorophenol contamination of a stream leading into a 15-ha man-made lake. A fish kill occurred immediately after the spill. Water samples taken 2 to 17 months after the spill contained 6 to 19 μg/liter pentachlorophenol. Pentachlorophenol levels in water increased after heavy rainfall, indicating continuous influx from contaminated watershed areas. Pentachlorophenol levels in sediment ranged from 2 to 1300 μg/kg, with levels decreasing with time. Residues in fish decreased from 2.5 mg/kg to trace levels with 10 months. Levels in fish increased to 0.65 mg/kg following the period of heavy rainfall and recontamination and then decreased to trace levels in 6 months.

The contribution of municipal chlorination to pentachlorophenol levels in water supplies is unclear. Dougherty (1975, as cited by Arsenault, 1976) found 0.1 μg/liter pentachlorophenol in the Tallahassee, Florida, water supply. The source was not discussed, but it may have resulted from municipal chlorination of drinking water and subsequent chlorination of phenol to pentachlorophenol. Arsenault (1976) demonstrated that 10 mg/liter chlorine is capable of chlorinating 1 mg/liter phenol (from natural sources), yielding approximately 0.2 μg/liter pentachlorophenol. Considerable evidence indicates chlorination results in the formation of the lower chlorinated phenols, but the report by Arsenault (1976) is the only one suggesting that pentachlorophenol is generated by municipal chlorination.

Bevenue et al. (1972) studied pentachlorophenol levels in the Sand Island outfall in Hawaii. This sewage outfall, which receives all sewage from the Honolulu area, contained 2.6 μg/liter pentachlorophenol in a 24-hr composite discharge. It is not known whether the sewage effluent was treated prior to discharge.

Buhler, Rasmusson, and Nakaue (1973) found that pentachlorophenol levels in 24-hr composite samples of sewage effluent collected simultaneously from three Oregon cities ranged between 1 and 4 μg/liter. Water samples from the Willamette River contained between 0.10 and 0.70 μg/liter.

Significant concentrations of pentachlorophenol were found in water treated at the Taylor Water Treatment Plant in Corvallis; 0.3 μg/liter pentachlorophenol of unknown source was found in the treated drinking water. The pentachlorophenol concentrations in the Willamette River were at least ten times greater than calculated values derived by assuming that the only source of pentachlorophenol was municipal sewage. Industrial sources could be responsible for this discrepancy.

D.7.2.2.2 Nonpoint Sources — The diverse nonpoint sources of pentachlorophenol in water can be divided into two general types: (1) pentachlorophenol pollution of the environment over wide areas, such as use of molluscicides for the control of snail populations, algicides, preemergence herbicides, or defoliants for cotton and soybeans (Higginbotham, Ress, and Rocke, 1970), and (2) leaching from objects treated with pentachlorophenol for preservative purposes (e.g., bactericides or fungicides). In the absence of microbiological breakdown of the compound, pentachlorophenol-treated lumber may be a continuing nonpoint source of pollution. Because pentachlorophenol is widely used in urban and industrialized areas, this nonpoint source may become a point source if it reaches storm sewers and is discharged into a stream.

The contribution of nonpoint pollution to the total environmental load is difficult to assess. Pentachlorophenol has limited use in the United States as a herbicide, molluscicide, and algicide, but in other countries pentachlorophenol use has continued unabated. Death of wildlife in Surinam (Vermeer et al., 1974) was attributed to the use of pentachlorophenol as a molluscicide in the rice fields (Section D.5.1.2.2.3). Fish kills have been reported in Korea (Shim and Self, 1973) and Japan (Nitta, 1972) as a result of pentachlorophenol use in rice fields (Section D.5.2.2.3). Thus, widespread pentachlorophenol contamination can result in serious environmental hazards. Vermeer et al. (1974) suggested that the use of pentachlorophenol as a molluscicide should be reexamined and perhaps banned because of its toxicity to wildlife.

Substantial levels of pentachlorophenol have been found in the rivers of Japan (Goto, 1971). Residues in river water in southwestern Japan ranged from 0.01 to 10 μg/liter in July 1969 (Section D.6.2.2.5). Goto (1971) stated that these concentrations are not ecologically dangerous, but this opinion is questionable, particularly when one considers the evidence that pentachlorophenol may bioaccumulate in aquatic organisms. Zitko, Hutzinger, and Choi (1974) surveyed pentachlorophenol levels in the aquatic fauna in New Brunswick, Canada (Table D.7.4). Although the levels were not toxicologically significant, detectable amounts were found in aquatic fauna from "a relatively clean area." The source was unclear.

D.7.2.3 Distribution in Soil

Ambient pentachlorophenol levels in soil are not available. Pentachlorophenol is resistant to degradation, and a persistence ranging from two weeks to one year has been reported (Section D.7.3.3). In general, the assumption is made that when pentachlorophenol is applied to land as

TABLE D.7.4. CONCENTRATION OF PENTACHLOROPHENOL IN AQUATIC FAUNA AND COMMERCIAL FISH FOOD IN NEW BRUNSWICK, CANADA

Sample	Pentachlorophenol content (ng/g wet wt)
Cod	0.82
Winter flounder	1.77 3.99
Sea raven	<0.5
Silver hake	1.75
Atlantic salmon	1.26 0.54
White shark liver	10.83
Double-crested cormorant egg	0.36
Herring gull egg	0.51
Fish food	2.23

Source: Adapted from Zitko, Hutzinger, and Choi, 1974, Table 1, p. 651. Reprinted by permission of the publisher.

a herbicide or desiccant, the initial level of pentachlorophenol equals the amount applied.

Because pentachlorophenol is a synthetic molecule with no known biological function under normal conditions, it is reasonable to assume that the presence of pentachlorophenol in soil results from man's activities. An exception to this assumption is the production of pentachlorophenol as a metabolic product of other substances. The formation of pentachlorophenol from hexachlorobenzene has been suggested in the monkey and the rat, and pentachlorophenol has been detected in rabbit urine following the administration of lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane) (Section D.6.2.2.5). Lu and Metcalf (1975) confirmed the formation of pentachlorophenol in a model aquatic ecosystem following the administration of hexachlorobenzene (Section D.5.2.1.3).

D.7.3 ENVIRONMENTAL FATE

Pentachlorophenol is an organic molecule and its ultimate fate under aerobic conditions should be degradation to chlorine, carbon dioxide, and water; anaerobically, several products are possible. An evaluation of the chemical properties of the compound (Section D.2) and monitoring information would place the compound in the category of persistent compounds; however, under appropriate conditions, pentachlorophenol is biodegradable. Conditions promoting pentachlorophenol degradation in the environment and in model laboratory systems are discussed in this section.

D.7.3.1 Mobility and Persistence in Air

Only limited data are available on the mobility and persistence of pentachlorophenol in air. It may reach levels which are toxic to humans, animals, and plants (Ferguson, 1959; Plakhova, 1966; Anonymous, 1970). Toxic effects in greenhouse experiments indicate that pentachlorophenol was carried by air (Ferguson, 1959). Information on the transport and persistence of pentachlorophenol volatilizing from treated objects is not available. Substantial levels of pentachlorophenol are present in rainwater in areas where pentachlorophenol is widely used (Bevenue, Ogata, and Hylin, 1972). The prime mechanism of removal of pentachlorophenol from the atmosphere is likely to be washout during precipitation.

D.7.3.2 Mobility and Persistence in Aquatic Environments

Pentachlorophenol (and/or sodium pentachlorophenate) may be present in aquatic environments in one (or more) of the following forms: (1) dissolved in either free or complexed form, (2) sorbed to suspended and/or bottom sediments, or (3) carried in biological tissues. Movement of pentachlorophenol in water depends primarily on hydrological factors such as currents and, in the case of transportation by organisms, movement and migration of the organism. In studies with DDT, Vind, Muraoka, and Mathews (1973) showed that the water-insoluble, organochlorine insecticides may be transported in seawater primarily by absorption in the fatty cell membranes of microorganisms. The extent of pentachlorophenol mobility due to transportation by aquatic organisms warrants further intensive investigation.

The persistence of pentachlorophenol in aquatic environments depends on several environmental variables, but the interrelationships have not been fully characterized. Pentachlorophenol may be effectively removed from the aqueous phase by (1) volatilization into the atmosphere, (2) photoinactivation, (3) adsorption, or (4) biodegradation. Hilton, Yuen, and Nomura (1970) demonstrated in laboratory experiments that the pentachlorophenol content of aerated solutions decreases rapidly, presumably by volatilization. The contribution of this phenomenon to pentachlorophenol removal from water is unclear. In general, decomposition of pentachlorophenol in clear, shallow-water areas is rapid; longer persistence times are seen in deep, turbid waters. Adsorption to sediments is an important factor in pentachlorophenol removal from water (Section D.2.5.3). Decomposition of pentachlorophenol in aqueous solutions has been demonstrated under laboratory conditions with suitably activated microbial populations (Section D.7.3.5). In the laboratory, microorganisms contained in activated sludge derived from sewage treatment plants exposed to pentachlorophenol are capable of degrading pentachlorophenol; however, the ubiquity of these microorganisms in the environment remains an open question.

Interactions among pentachlorophenol, soil, and water are made complex by many variables. The system has been simplified in experimental studies by maintaining some of the factors at a constant level. However, some variables such as microbial activity, photodecomposition, volatility, adsorption-desorption equilibria, soil pH, organic matter in soils, and lateral movement by water are difficult, if not impossible, to control. The interactions among these variables as they relate to pentachlorophenol decomposition in a particular situation have not been quantified. Furthermore, pentachlorophenol may be transported by organisms in the water.

Under certain specific conditions, pentachlorophenol levels in water declined to negligible amounts in 48 hr (Bevenue and Beckman, 1967), but other experiments indicated that substantial amounts of pentachlorophenol may remain in a laboratory system for more than 120 days (Strufe, 1968). Thus, depending on environmental conditions, pentachlorophenol may degrade rapidly and pose little or no environmental hazard, or it may have marked longevity and thereby pose a potential hazard of indeterminate proportions. Increased emphasis should be placed on isolating the factors primarily responsible for controlling the fate and longevity of pentachlorophenol in various segments of the environment.

D.7.3.3 Mobility and Persistence in Soil

The mobility and persistence of pentachlorophenol in soils depend on the physical and chemical characteristics of the soil as well as the prevailing microbiological population. The effect of soil characteristics on the mobility and persistence of pentachlorophenol have been discussed previously (Section D.2.5.3). Because these same factors control the rate of biological degradation of pentachlorophenol, they are also reviewed in this section.

The mobility of pentachlorophenol in soils is affected by complex environmental variables such as soil characteristics and climate. The degree of mobility depends to a large extent on the tenacity with which the compound is adsorbed by soil particles (Section D.2.5.3). The persistence of pentachlorophenol has been correlated with many factors, including soil temperature, moisture content, soil type, content of organic matter and free iron oxides, type and content of clay minerals, sorptivity, and cation-exchange capacity. The complex interrelationships among these factors preclude an accurate prediction of the rate of pentachlorophenol degradation in various segments of the environment, although pentachlorophenol degradation studies have been conducted under laboratory conditions. Some general conclusions can be obtained from the literature.

Loustalot and Ferrer (1950) determined that the degree of pentachlorophenol inactivation (to corn and cucumbers) increases with increased temperature. Pentachlorophenol vaporizes from treated wood, producing a toxic effect on nursery seedlings grown in close proximity (Ferguson, 1959). The effect of temperature on the vaporization of pentachlorophenol from soil has not been studied; however, vaporization is a likely mechanism for its dissipation from soil, and high temperature may contribute to increased vaporization.

The moisture content of soil also appears to affect the degradation rate. Young and Carroll (1951) noted that pentachlorophenol degradation was optimum when the moisture content of the soil approximated the moisture equivalent. Kuwatsuka and Igarashi (1975) reported that degradation of pentachlorophenol was faster under flooded conditions (250% water content) than under upland conditions (60% water content). Loustalot and Ferrer (1950) studied the degradation of pentachlorophenol in fertile, sandy loam and found that sodium pentachlorophenate was relatively stable in air-dried soil, persisted for two months in soil with moderate moisture content, and persisted for one month in water-saturated soil.

Soil composition has a marked effect on persistence of pentachlorophenol. Loustalot and Ferrer (1950) reported that pentachlorophenol persisted longer in heavy clay than in sandy or sandy clay soils. Kuwatsuka and Igarashi (1975) found a significant positive correlation between the pentachlorophenol degradation rate and the organic matter content of the soil. No degradation occurred in subsoil samples from forested areas after 50 days of incubation. Kuwatsuka and Igarashi (1975) also correlated rate of degradation with clay mineral composition, free-iron content, phosphate absorption coefficients, and cation-exchange capacity of the soil; a lower level of probability was found with these variables than with organic matter. Little or no correlation was found between degradation and soil texture, clay content, degree of base saturation, soil pH, or available phosphorus content (Table D.7.5).

Microbiological activity plays a primary role in the degradation of pentachlorophenol in soil. Young and Carroll (1951) stated that pentachlorophenol decays more rapidly when the ambient temperature approaches the optimal value for microbiological activity, indicating that a microbiological mechanism for degradation exists. They also demonstrated that decomposition occurred more rapidly in soils with high organic matter content. Ide et al. (1972) found no decay in sterilized soil samples. The significant and positive correlation between organic matter content and pentachlorophenol degradation as well as the fact that degradation did not occur over a 50-day period in a forest subsoil sample devoid of organic matter led Kuwatsuka and Igarashi (1975) also to suggest that microorganisms play an important role.

Conversely, Ide et al. (1972) found that pentachlorophenol degraded faster in an immature paddy soil with low organic matter content than in an immature paddy soil with high organic matter content. Mature paddy soils may contain microbiological populations which are acclimated to pentachlorophenol as a result of its regular use in rice fields, which would explain the discrepancy between these findings and those of Kuwatsuka and Igarashi (1975). Kuwatsuka and Igarashi (1975) studied widely different soils from flooded and upland areas of Japan. In the laboratory, pentachlorophenol degraded most rapidly when upland soils were maintained in an aerated condition. When the soils were flooded, the rate decreased. In soil samples from flooded areas, pentachlorophenol degraded most rapidly in the flooded state and at a slower rate under aerated conditions. Thus, pentachlorophenol-degrading microorganisms present in upland or paddy soils survived transfer to the laboratory and

TABLE D.7.5. SIMPLE CORRELATION COEFFICIENTS RELATING DEGRADATION RATE OF PENTACHLOROPHENOL TO PROPERTIES OF SOILS[a]

Soil property	Correlation coefficient after incubation for			
	5 days	10 days	20 days	30 days
	Flooded conditions			
Total carbon content	0.839^b	0.815^b	0.625^c	0.827^b
Clay content	0.182	0.457	0.352	0.432
Cation-exchange capacity	0.831	0.574^d	0.599^d	0.525^d
Available phosphorus content	0.392	0.365	0.499^d	0.589^d
Free iron content	0.889^b	0.633^d	0.858^b	0.409
pH (H_2O)	0.392	0.630^d	0.420	0.432
Phosphate absorption coefficient	0.877^b	0.730^d	0.908^b	0.922^b
	Upland conditions			
Total carbon content	0.817^c	0.814^c	0.539^d	0.383
Clay content	0.046	0.264	0.194	0.152
Cation-exchange capacity	0.926^c	0.763^c	0.368	0.333
Available phosphorus content	0.675^d	0.642^d	0.144	0.198
Free iron content	0.091	0.597	0.358	0.387
pH (H_2O)	0.184	0.539	0.444	0.153
Phosphate absorption coefficient	0.937^b	0.821^c	0.368	0.253

[a] Degradation rate = $\dfrac{\text{applied amount} - \text{residual amount}}{\text{applied amount}} \times \dfrac{1}{\text{incubation period}}$.

[b] Significant at the 0.1% level of probability.

[c] Significant at the 1% level of probability.

[d] Significant at the 5% level of probability.

Source: Adapted from Kuwatsuka and Igarashi, 1975, Table 2, p. 410.

were most active when placed in environments to which they were adapted. Alternatively, pentachlorophenol may be degraded by the several bacterial species capable of surviving in either upland or flooded conditions.

The persistence of pentachlorophenol in soil is summarized in Table D.7.6. The time ranges from 21 days to several hundred days; thus, pentachlorophenol is moderately persistent under some conditions and more persistent under others. A moderate to high organic matter content and an acclimated microbial population facilitate pentachlorophenol decomposition in soil. Under conditions unfavorable for bacterial growth, degradation is extremely slow.

D.7.3.4 Mobility and Persistence in Treated Wood

Little information is available on the mobility and persistence of pentachlorophenol in preservative-treated items other than wood. Wood preservation accounts for most of the pentachlorophenol use in the United States; therefore, its fate and persistence in treated wood is of major importance. Environmental contamination from other lesser uses have been largely ignored. The mechanism of pentachlorophenol depletion from treated wood has been studied extensively. Dissipation from and migration within the wood depend on many factors such as the type of wood and its moisture content, the solvent accompanying the pentachlorophenol, the treatment process, and the soil conditions and climate where the wood is used (Arsenault, 1970; Leutritz, 1971; Walters and Arsenault, 1971; Gjovik, Bendtsen, and Roth, 1972). Information on these factors is useful in determining the optimum conditions for maximum longevity of the wood, but it provides little insight into the environmental impact of pentachlorophenol-treated wood.

Because pentachlorophenol is a very effective wood preservative, it can be inferred that pentachlorophenol dissipation is not substantial when wood is treated properly. Maximum retention of the preservative is a primary characteristic of its effectiveness. Utility poles treated with pentachlorophenol survived for 25 years without substantial decay (Arsenault, 1970). Arsenault (1976) stated, "The fact that PCP-oil solution moves down the pole towards the groundline is well established. In the case of an occasional bleeding pole, the groundline and adjoining soil can get saturated with PCP-oil solution. Therefore, it is reasonable to ask what are the environmental effects of this migration. Generally speaking, the vast majority of the poles in service have vegetation growing adjacent to the pole surface indicating no significant environmental effect; PCP is an effective herbicide, being phytotoxic. We have shown that PCP and the OCDD (octachlorodibenzo-*p*-dioxin) are both biodegraded by soil if any soil contamination does occur. There is no significant migration through the soil." The pentachlorophenol content of soil adjacent to treated utility poles was determined by Arsenault (1976). Soil samples were taken at various distances from treated utility poles and to depths of 6 in. at the base of 30 treated poles in six widely scattered locations in the United States (Table D.7.7). Soil at 1 in. from the poles contained an average pentachlorophenol content of 658 mg/kg; the highest value found was 9500 mg/kg. Twelve inches from the treated poles

TABLE D.7.6. PERSISTENCE OF PENTACHLOROPHENOL IN SOIL

Degradation parameter	Soil type	Conditions	Time	Source
90% degradation	Arable layer in rice fields (11 soils)	60% water	Approx 50 days	Kuwatsuka and Igarashi, 1975
		250% water	Approx 30 days	
	Forest red-yellow soil sublayer	60% water	No degradation in 50 days	Kuwatsuka and Igarashi, 1975
		250% water	No degradation in 50 days	
	Wooster silt loam	7.5 kg/ha pentachlorophenol, optimum conditions for microbial growth	Approx 22 days	Young and Carroll, 1951
	Mature paddy soil	Low organic content	1 month	Ide et al., 1972
98% degradation	Permeable soil	Composted with sludge from wood-treating plant	205 days	Arsenault, 1976
Complete degradation	Dunkirk silt loam	Aerated, aqueous soil suspension	Approx 72 days	Alexander and Aleem, 1961
	Paddy soil	Soil perfusion	21 days	Watanabe, 1973
Effect on growth of corn and cucumbers	Fertile sandy loam	Air-dried	>2 months	Loustalot and Ferrer, 1950
		Medium water	2 months	
		Water-saturated	1 month	

TABLE D.7.7. DETERMINATION OF PENTACHLOROPHENOL IN SOIL SURROUNDING PENTACHLOROPHENOL-TREATED UTILITY POLES

Location	Soil type and condition	Distance from pole		Pentachlorophenol content (mg/kg soil)
		(in.)	(cm)	
Jackson, Tennessee	Well-drained clay	1	2.54	1.4
		12	30.5	0.13
		60	152.4	0.04
	Well-drained clay	1	2.54	0.32
		12	30.5	0.05
		60	152.4	0.04
	Well-drained clay	1	2.54	120
		12	30.5	3.1
		60	152.4	0.81
Savannah, Georgia	Sandy, well-drained, grass and weeds next to pole	1	2.54	620
		12	30.5	0.08
		60	152.4	0.04
	Sandy, well-drained, grass and weeds next to pole	1	2.54	1.9
		12	30.5	0.05
		60	152.4	0.03
	Sandy, well-drained, black coating on soil within 6 in., no vegetation	1	2.54	1200
		12	30.5	29
		60	152.4	0.18
Spartanburg, South Carolina	Clay loam	1	2.54	620
		12	30.5	1.7
		60	152.4	0.5
	Clay loam	1	2.54	300
		12	30.5	0.34
		60	152.4	0.09
	Clay loam	1	2.54	50
		12	30.5	1.5
		60	152.4	0.08
	Clay loam	1	2.54	14
		12	30.5	0.78
		60	152.4	0.24
	Clay loam	1	2.54	34
		12	30.5	3.4
		60	152.4	0.11
Gresham, Oregon	Loam, nearest plant 4 in. from pole	1	2.54	380
		12	30.5	0.42
		60	152.4	0.30
	Loam, no grass within 3 in. of pole	1	2.54	5200
		12	30.5	40
		60	152.4	0.4
	Loam, no grass within 6 in. of pole on east side and 2 in. on west side	1	2.54	9500
		12	30.5	1.4
		60	152.4	0.2

(continued)

TABLE D.7.7 (continued)

Location	Soil type and condition	Distance from pole		Pentachlorophenol content (mg/kg soil)
		(in.)	(cm)	
Selma, Alabama	Sandy loam	1	2.54	5.2
		12	30.5	0.54
		60	152.4	0.34
	Sandy loam	1	2.54	16
		12	30.5	0.74
		60	152.4	0.44
	Sandy loam	1	2.54	12
		12	30.5	1.8
		60	152.4	0.60
Montgomery, Alabama	Sandy loam	1	2.54	7.4
		12	30.5	0.94
		60	152.4	1.0
	Sandy loam	1	2.54	4.8
		12	30.5	2.2
		60	152.4	0.48
	Sandy loam	1	2.54	12
		12	30.5	0.88
		60	152.4	0.30
Chico, California	Sandy loam, vegetation moderate around pole	1	2.54	0.72
		12	30.5	0.22
		60	152.4	0.16
	Sandy loam, grass around pole	1	2.54	1.3
		12	30.5	0.14
		60	152.4	0.22
	Sandy loam, light vegetation around pole	1	2.54	0.19
		12	30.5	0.16
		60	152.4	0.18
	Clay, heavy weeds around pole	1	2.54	0.22
		12	30.5	0.07
		60	152.4	0.09
	Clay, heavy vegetation around pole	1	2.54	0.42
		12	30.5	0.22
		60	152.4	0.26
Paradise, California	Clay, light grass to no vegetation	1	2.54	0.70
		12	30.5	0.20
		60	152.4	0.20

Source: Adapted from Arsenault, 1976, Tables 6-11, pp. 16-19. Reprinted by permission of the publisher.

the soil had an average content of 3.4 mg/kg, with a high value of 40 mg/kg. At 5 ft from the poles, pentachlorophenol concentrations averaged 0.26 mg/kg, which closely approximated the values obtained in unexposed samples (0.2 to 0.4 mg/kg). This study indicates that leaching from preservative-treated wood does not result in widespread contamination of the surrounding soil. Biodegradation in the soil may account for the gradually decreasing amounts of pentachlorophenol with greater distance (Arsenault, 1976); also, some biodegradation may occur in the treated wood.

Pentachlorophenol depletion in treated wood was also studied by Leutritz (1965). The pentachlorophenol content of treated pine strips exposed to sterile and nonsterile soils decreased by 22% to 27% over a 50-day incubation period. Because no differences were found between the sterile and nonsterile environments, the author concluded that leaching rather than biodegradation was responsible for pentachlorophenol depletion.

Other investigators have found that biodegradation of pentachlorophenol does occur in treated wood. Unligil (1968) demonstrated that a common wood-inhabiting fungus (*Trichoderma viride*) and a wood-decaying fungus (*Coniophora puteana*) could metabolize pentachlorophenol; 62% of the pentachlorophenol from samples containing 5.8 kg/m^3 was dissipated in 52 days. Duncan and Deverall (1964) also demonstrated pentachlorophenol degradation by common wood-inhabiting fungi. The ability of three species of *Trichoderma* to degrade pentachlorophenol was demonstrated by Cserjesi (1967). One species, *Trichoderma virgatum*, degraded pentachlorophenol by forming pentachloroanisole (Cserjesi and Johnson, 1972). Methylation of pentachlorophenol by fungi was also reported by Curtis et al. (1974). Three species found commonly in poultry litter — *Scopulariopsis brevicaulis*, *Aspergillus sydowi*, and *Penicillium crustosum* — catalyzed the methylation reaction. Pentachloroanisole was not the sole metabolic product because in one test system only 10% to 20% of the original pentachlorophenol was present as pentachloroanisole, despite the fact that no pentachlorophenol was detected following degradation (Cserjesi and Johnson, 1972). Pentachloroanisole may be the first step in the biodegradation of pentachlorophenol, or another pathway which does not include pentachloroanisole as a product may exist.

In summary, pentachlorophenol is moderately stable in preservative-treated wood and is slowly dissipated as a result of biodegradation and leaching. The environmental hazard is probably minimal. Soil contamination is probably confined to very localized areas surrounding the treated wood, and the pentachlorophenol which reaches the soil is degraded rapidly enough to prevent any hazard to plants or animals.

D.7.3.5 Microbial Decomposition in Soils and Water

Pentachlorophenol is considered to be a refractory compound of the chlorophenol family. Bacteria capable of degrading pentachlorophenol were not isolated until Chu and Kirsch (1972) used continuous-flow enrichment to isolate a bacterial culture (designated KC-3) capable of metabolizing pentachlorophenol as a sole source of organic carbon and energy. The morphological and physiological characteristics of KC-3 suggested a relationship to the saprophytic coryneform bacteria. Chu and Kirsch (1973) established that pentachlorophenol metabolism by KC-3 was highly responsive to enzyme induction with pentachlorophenol as the inducer. Partial induction of the pentachlorophenol-degrading system occurred when 2,4,6-trichlorophenol was used as an inducer. KC-3 can degrade pentachlorophenol (200 mg/liter) in a complex organic medium, indicating that pentachlorophenol metabolism is not precluded by the presence of other nutrient substances (Hayes and Kirsch, 1973). Degradation products of pentachlorophenol metabolism by KC-3 were not characterized.

A microbial population capable of rapid pentachlorophenol degradation was found in a soil sample from the grounds of a wood-products manufacturer in Terre Haute, Indiana (Kirsch and Etzel, 1973). This population was grown in media to which increasing amounts of pentachlorophenol were added for three months. When the microbes were fully acclimated (25 days), they were dosed with 100 mg/liter pentachlorophenol; 68% was degraded in 24 hr. The culture was most effective when pentachlorophenol was the sole source of carbon. When cells were grown in nutrient broth containing pentachlorophenol (i.e., pentachlorophenol was not the sole carbon source), oxidation was retarded by a factor of 2. Thus, optimum conditions for growth of the organism are not necessarily optimum for degradation of pentachlorophenol. Over a 60-day acclimation period, the oxidation capacity of the culture increased initially and then rapidly declined (Table D.7.8). The reason for this decline is not known. The authors stated, "The data might be interpreted to mean that the PCP-oxidizing organisms, although slower growing than other organisms in the culture, gradually become a significant fraction of the population as the result of some selective culture advantage that may have been related to the gradually increasing concentration of exogenous PCP. The reason for the sharp decrease in PCP-oxidizing capacity is not known."

Microbial degradation of pentachlorophenol was also identified by Kuwatsuka and Igarashi (1975) in a bacterial culture derived from a paddy soil. The degradation products were 2,3,4,5-, 2,3,4,6-, and 2,3,5,6,-tetrachlorophenol; 2,3,4-, 2,4,5-, 2,3,5-, 2,3,6-, and 2,4,6-trichlorophenol; and pentachlorophenol methyl ether. Principal products were pentachlorophenol methyl ether, 2,3,4,5-tetrachlorophenol, and 2,3,6- and 2,4,6-trichlorophenol. Ide et al. (1972) found many of the same degradation products in paddy soils. The specific products identified were 2,3,4,5-, 2,3,5,6-, and 2,3,4,6-tetrachlorophenol; 2,4,5- and 2,3,5-trichlorophenol; 3,4- and 3,5-dichlorophenol; and 3-monochlorophenol. Pentachlorophenol methyl ether was not detected.

Watanabe (1973) reported pentachlorophenol degradation in soil samples perfused with 40 mg/liter pentachlorophenol, but no degradation products were characterized. Bacterial isolates were derived from a soil perfusion enrichment culture. After two to three weeks incubation at 30°C, degradation and complete dechlorination of pentachlorophenol were caused by *Pseudomonas* sp. or a bacterium from a closely related genera. Degradation was most efficient in a medium containing pentachlorophenol

TABLE D.7.8. OXIDATION OF PENTACHLOROPHENOL BY A HETEROGENEOUS ACCLIMATING BIOMASS[a]

Sampling time (days)	CO_2 formed (dpm/day)	Oxidative capacity[b] (%)
1	4,400	10
5	5,100	11
7	9,800	22
12	20,000	44
20	17,000	38
25	31,000	68
32	31,000	68
39	29,000	65
42	27,000	61
47	17,000	38
53	4,000	9
57	50	1

[a] During this acclimation cycle a 4-liter completely mixed, aerated reactor received 40 mg/day pentachlorophenol and 500 mg/day nutrient broth.

[b] Percent radioactive pentachlorophenol carbon converted to CO_2 in 24 hr.

Source: Adapted from Kirsch and Etzel, 1973, Table IV, p. 362. Reprinted by permission of the publisher.

and inorganic salts. When the organism was cultured on glucose bouillon agar containing pentachlorophenol, growth was slower and pentachlorophenol was not degraded. Thus, pentachlorophenol is an adequate, but not a preferred, substrate for this bacterium.

Cserjesi (1972) used gas chromatography to tentatively identify tetrochlorodihydroxyphenols and their monomethyl ethers as metabolic products of pentachlorophenol by *Aspergillus* sp.; no dimethyl ethers were detected. A soil bacterium isolated by Suzuki and Nose (1971, as cited by Cserjesi, 1972) degraded pentachlorophenol. The major metabolite was pentachloroanisole; tetrachlorohydroquinone dimethyl ether was identified as a minor metabolite.

Investigations by Arsenault (1976) demonstrated pentachlorophenol degradation by microorganisms in soil. Pentachlorophenol-containing sludges from commercial wood-treating operations were composted with a "permeable" soil. Quantitative degradation of pentachlorophenol occurred in 205 days if the pentachlorophenol concentration did not exceed 200 μg/g. In another experiment, process water from a wood-treating plant containing 35 mg/liter pentachlorophenol was percolated through soil columns in the laboratory. Over a 2.5-year period, effluent water from 1-m columns consistently contained less than 1 mg/liter pentachlorophenol. Also, pentachlorophenol-containing sludges were composted with soil at concentrations of 100, 200, and 300 mg/liter, and distilled water was added weekly to simulate rainfall. After 205 days, the soil and leachates were analyzed for pentachlorophenol and only negligible amounts were found (Table D.7.9). The soil was contained in 20-cm glass vessels to approximate topsoil cover in the field.

TABLE D.7.9. LEACHING OF PENTACHLOROPHENOL FROM "PENTA SLUDGES" IN SOIL IN 205 DAYS

Leachate volume (ml)	Pentachlorophenol in leachate		
	(mg/liter)	(mg)	(% of original pentachlorophenol)
3597	0.019	0.070	0.0048
3459	0.067	0.23	0.0061
3712	0.108	0.40	0.0070

Source: Adapted from Arsenault, 1976, Table 13, p. 20. Reprinted by permission of the publisher.

Watanabe (1977) demonstrated in field plot studies that within six weeks after initial application of pentachlorophenol, pentachlorophenol-decomposing microorganisms increased by three orders of magnitude. Some specificity was noted; soils receiving pentachlorophenol were not enriched with tetrachlorophenol-decomposing microorganisms.

In summary, bacteria and fungi capable of degrading pentachlorophenol exist in the environment, but these microorganisms may not be widespread. In most cases of rapid pentachlorophenol degradation by microorganisms, the source of the initial microbial inoculum was an area where pentachlorophenol had been used for a considerable time. For example, microorganisms capable of degrading pentachlorophenol were derived from soil cultures from wood preserving plants where the soil was frequently saturated with pentachlorophenol. Other inocula were derived from paddy fields where pentachlorophenol was widely used as a herbicide and from sewage treatment facilities exposed to high inputs of pentachlorophenol. In other cases, enrichment cultures were exposed to gradually increasing amounts of pentachlorophenol. Bacteria may or may not possess qualities which allow them to compete effectively in a mixed population. Watanabe (1973) and Kirsch and Etzel (1973) reported that pentachlorophenol-degrading bacteria may not be optimally efficient in the presence of alternative substrates such as nutrient broth. However, the bacteria isolated by Chu and Kirsch (1972), KC-3, degraded pentachlorophenol in the presence of other nutrients. Thus, some bacteria may have the capability of degrading pentachlorophenol in the presence of other organic nutritional sources. Metabolic pathways for pentachlorophenol degradation and the environmental fate of pentachlorophenol metabolites are areas for further investigation.

D.7.3.6 Photodecomposition

The photochemistry of pentachlorophenol and other chlorinated phenolic herbicides has been widely investigated. Model studies have shown that aqueous solutions of pentachlorophenol or its salts are subject to photodecomposition, and this observation has been reinforced by practical experience in the field.

D.7.3.6.1 Mechanism -- Photochemical reactions of halogenated aromatic compounds appear to follow free-radical pathways (Plimmer, 1970). Because of the free-radical character of the reaction, the structure of the products depend on the properties of the solvent. Water is the solvent involved in the environmental photochemical reactions; therefore, studies of photochemical degradation in aqueous solutions are particularly pertinent. Results of photochemical reactions in nonaqueous solvents should be carefully examined for applicability to environmental conditions.

The absorbance spectrum of phenols undergoes a characteristic bathochromatic shift with change from the protonated form to the anion. Because the wavelength of light absorbed is affected, it is reasonable to assume that there are changes in photochemical behavior as well. According to Plimmer (1970), the nature of the reaction products depends on the wavelength of light absorbed. Another factor which influences the spectrum of a molecule is the surfaces with which it is associated in the environment, or more specifically, bonding interactions with the surface. Interactions which affect the reaction products include simple adsorption, coulombic forces, van der Waals forces, and charge transfer. In short, the chemical environment of the molecule profoundly influences its photochemical behavior. Unfortunately, there have been relatively few studies of pesticide photochemistry on environmental surfaces (Plimmer, 1970). Adsorption on silica does cause a shift in the ultraviolet spectra, which provides evidence for hydrogen bonding of the phenol. Aqueous solutions of sodium pentachlorophenate decomposed when exposed to sunlight, as evidenced by a color change from clear to purple after about ten days (Munakata and Kuwahara, 1969).

Hamadmad (1967) characterized some of the breakdown products of pentachlorophenol in various solutions. Ultraviolet irradiation of pentachlorophenol in hexane or MeOH gave 2,3,5,6-tetrachlorophenol, presumably by reductive dechlorination. However, an aqueous suspension of pentachlorophenol produced little tetrachlorophenol when irradiated; polymeric substances were the major photolysis products. Crosby and Hamadmad (1971) concluded that 2,3,5,6-tetrachlorophenol was the major breakdown product of pentachlorophenol photolysis, but they used only organic solvents and no aqueous solutions. Munakata and Kuwahara (1969) studied the photochemical reaction products obtained when an aqueous solution of 20 g/liter sodium pentachlorophenate was irradiated. After ten days of sunshine, 50% of the sodium pentachlorophenate was lost. The chemical structures of the degradation products are shown in Figure D.7.1.

D.7.3.6.2 Effect of Photodecomposition — Hiatt, Haskins, and Olivier (1960) recognized that photochemical degradation may be a factor which

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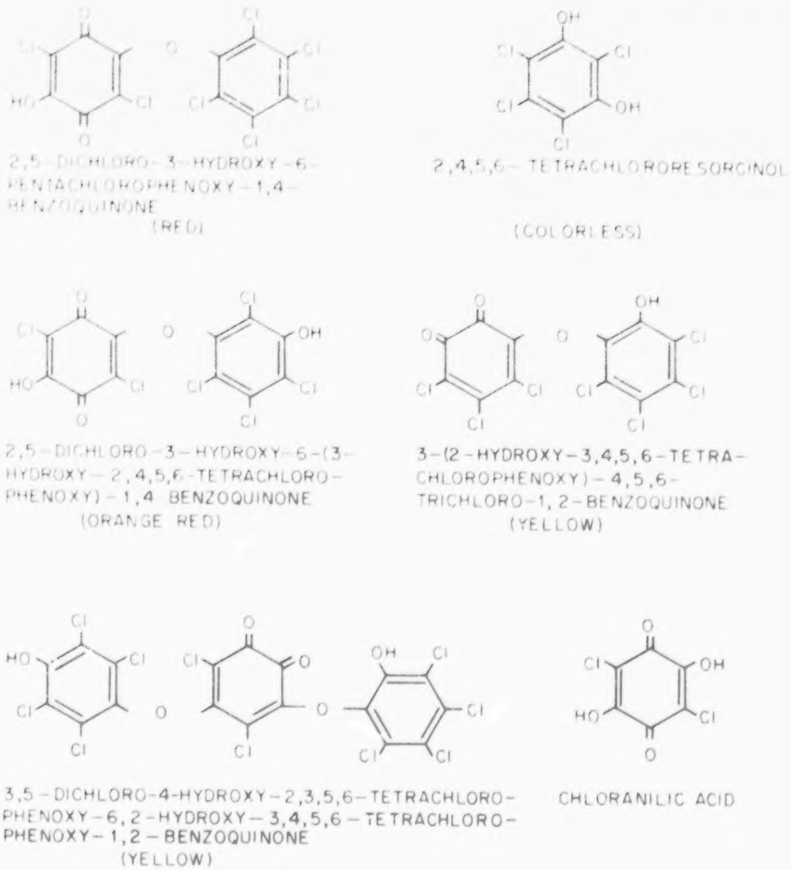

2,5—DICHLORO—3—HYDROXY—6—PENTACHLOROPHENOXY—1,4—BENZOQUINONE
(RED)

2,4,5,6—TETRACHLORORESORCINOL
(COLORLESS)

2,5—DICHLORO—3—HYDROXY—6—(3—HYDROXY—2,4,5,6-TETRACHLOROPHENOXY)—1,4 BENZOQUINONE
(ORANGE RED)

3—(2—HYDROXY—3,4,5,6—TETRACHLOROPHENOXY)—4,5,6—TRICHLORO—1,2—BENZOQUINONE
(YELLOW)

3,5—DICHLORO-4-HYDROXY—2,3,5,6—TETRACHLOROPHENOXY—6,2—HYDROXY—3,4,5,6—TETRACHLOROPHENOXY—1,2—BENZOQUINONE
(YELLOW)

CHLORANILIC ACID

Figure D.7.1. Photochemical degradation products obtained from irradiation of an aqueous solution of sodium pentachlorophenate. Source: Adapted from Munakata and Kuwahara, 1969, Figure III, p. 17. Reprinted by permission of the publisher.

reduces the efficacy of pentachlorophenol on its target organism. They reported field observation of unexpectedly poor control of the snail vectors of schistosomiasis in South African streams. These streams were all small, shallow, and rapidly flowing and were exposed to full sunlight during the day. The water was exceptionally clear. They speculated that photochemical destruction of sodium pentachlorophenate was the cause of the poor control. Results of a laboratory study by Hiatt, Haskins, and Olivier (1960) confirmed this hypothesis (Table D.7.10). The lethal effects of irradiated and control solutions were not significantly different. Thus, the decrease in sodium pentachlorophenate concentration in the irradiated samples, as measured by chemical methods, was accompanied by a decline in molluscicidal effects, which indicates that the decomposition products are nontoxic to snails.

TABLE D.7.10. SNAIL EGGS KILLED WITHIN 24 hr BY IRRADIATED AND UNTREATED SOLUTIONS WHEN EACH SOLUTION WAS DILUTED TO THE SODIUM PENTACHLOROPHENATE CONCENTRATION

Sodium pentachlorophenate concentration (mg/liter)	Irradiated solution		Untreated solution	
	Number of eggs	Eggs killed (%)	Number of eggs	Eggs killed (%)
0.12	68	0	75	5
0.20	66	73	80	76
0.25	182	88	130	82
0.34	90	92	74	100
0.5	160	89	107	100
1.0	116	98	128	100

Source: Adapted from Hiatt, Haskins, and Olivier, 1960, Table 2, p. 529. Reprinted by permission of the publisher.

The photochemical breakdown products discussed by Munakata and Kuwahara (1969) (Figure D.7.1) were tested for toxicity. The degradation products showed stronger fungicidal effects but weaker phototoxicity and fish toxicity than pentachlorophenol. Fungicidal effects were tested on leaf blight (*Zanthomonas oryzae*), rice blast (*Piricularia oryzae*), pear black spot (*Alternaria kikuchiana*), and grape ripe rot (*Glomerella cingulata*). Phytotoxic effects were tested on seeds of rice and rape. Fish toxicity was examined using Japanese killifishes (*Oryzias latipes*).

D.7.4 WASTE MANAGEMENT

Primary waste treatment processes are expected to be equally efficient with wastewater containing high amounts of pentachlorophenol (e.g., from wood preserving plants) or wastewaters containing low concentrations of pentachlorophenol (e.g., municipal sewage). Biological treatment of wastewater, on the other hand, requires the presence of acclimated populations of bacteria capable of degrading pentachlorophenol, which is more likely when a constant high level of pentachlorophenol is present (e.g., treatment of wastewater from wood preserving operations).

D.7.4.1 Primary Treatment

Primary treatment of wastewater involves the removal of particulate substances and emulsified oils. The effect of this type of treatment on the pentachlorophenol content of wastewater is probably minimal. Pentachlorophenol dissolved in oil is removed by this process, but pentachlorophenol dissolved in water is not. Because pentachlorophenol is highly soluble in organic solvents, its removal from wastewater from wood preserving operations may be substantial. Sodium pentachlorophenate, however, is highly soluble in water, and its removal by flocculation treatments is unlikely. Thompson and Dust (1971) treated wastewater samples containing pentachlorophenol with various amounts of lime and monitored the pentachlorophenol concentration. The wastewater contained substantial amounts of oils. The residual pentachlorophenol concentrations were reduced from 150 to 17 mg/liter with application of 2 g/liter of lime. The solubility of pentachlorophenol in water is approximately 17 mg/liter; thus, pentachlorophenol removal was assumed to be a result of flocculation of pentachlorophenol entrained in the oil. Wastewaters which do not contain oil probably do not show substantial reduction of pentachlorophenol levels unless the pentachlorophenol (or sodium pentachlorophenate) is adsorbed to sediment particles.

D.7.4.2 Secondary Treatment

Secondary treatment involves the removal of organic matter from wastewater by biological processes. Biological oxidation is widely used in processing domestic and industrial wastes. This method depends on the ability of microbial populations to degrade organic waste with the formation of nontoxic metabolites such as carbon dioxide and water. A number of different methods are used — trickling filtration, activated sludge, oxidation lagoons, and soil irrigation, as well as many variations of these basic methods. The effectiveness of trickling filtration and soil irrigation as biological treatment methods for pentachlorophenol has not been reported.

Evidence exists that pentachlorophenol-containing effluents can be degraded in an aeration lagoon (Sidwell, 1971). Pentachlorophenol was added to aeration lagoon influent, and the aerated solution was continuously analyzed for pentachlorophenol. In separate experiments, the pentachlorophenol concentration decreased from 39.5 to 0.5 mg/liter in three days and from 81 to 0.6 mg/liter in 30 hr. Thus, based on one laboratory study, the aeration lagoon is a reasonably efficient process for the secondary treatment of wastewater containing pentachlorophenol.

The effectiveness of activated sludge treatment on the pentachlorophenol content of wastewater is somewhat controversial. The ability of an activated sludge preparation to degrade various chlorophenols was studied by Ingols and Stevenson (1963). The sludge was acclimated to the presence of chlorophenols over a period of several months. Pentachlorophenol was the only chlorophenol tested which resisted degradation by the activated sludge population. In contrast, other investigators have demonstrated the ability of activated sludge preparations to degrade pentachlorophenol. Dust and Thompson (1973) studied the biodegradability of pentachlorophenol using bench-scale activated sludge units; removal efficiencies in excess of 90% were obtained. Waste from a wood preserving plant containing 23 mg/liter pentachlorophenol was treated successfully in the laboratory to produce an effluent containing 0.4 mg/liter pentachlorophenol (Kirsch and Etzel, 1973). Arsenault (1976) also found that activated sludge preparations obtained from wood preserving plants were capable of pentachlorophenol degradation. Thus, under specific laboratory conditions, activated sludge populations apparently are capable of degrading pentachlorophenol. The efficiency of this process must be assessed on an individual basis for each sewage treatment plant.

Of more serious concern is the effect of pentachlorophenol on activated sludge treatment when the compound is not a regular component of the influent wastewater. This situation could arise in municipal treatment plants subject to shock-loading conditions from nearby pentachlorophenol manufacturers or users (Section D.3.1.2).

D.7.4.3 Chemical Oxidation

Removal of phenols from municipal water supplies by chemical oxidation is routinely practiced because of the objectionable taste and odor even when they are present in relatively low concentrations. Chemical treatments involving the oxidation of phenols with chlorine, chlorine dioxide, ozone, and Fenton's reagent (H_2O_2 plus a ferrous iron salt) have been reported (Eisenhauer, 1964). Fenton's reagent does not attack pentachlorophenol. Vigorous oxidation of pentachlorophenol with nitric acid produces a mixture of tetrachloro-*o*-quinone and tetrachloro-*p*-quinone — a reaction exploited as a colorimetric analysis. It is unlikely that the less drastic oxidations used in waste treatment will go beyond the quinone stage and cause ring cleavage. If they do give oxidation products similar to those of the nitric acid reaction, they may reduce the toxicity of pentachlorophenol. Oxidation by ozone is a relatively new technique, and its effectiveness in degrading pentachlorophenol in wastewater has not been fully assessed.

Treatment of wastewater with chlorine or chlorine dioxide, a commonly used method, may be useful in treating wastewater from wood treatment plants. According to Thompson (1973), chlorination of pentachlorophenol results in the formation of chloranil, a compound which is much less toxic and more readily biodegradable than the parent chemical pentachlorophenol. However, chlorination of municipal wastes may cause more problems than it solves. Although Ingols and Ridenour (1948) reported that the oxidation of phenolic compounds by chlorine does not result in the formation of pentachlorophenol, more recent work (Arsenault, 1976) indicates that pentachlorophenol can be formed in municipal sewage treated with chlorine. Thus, under appropriate conditions, pentachlorophenol may be synthesized as a result of municipal chlorination of wastewater. This objection also applies to the chlorination of municipal drinking water supplies. Another more subtle hazard exists when chlorination is used for the treatment of wastewater or drinking water. Ingols, Gaffney, and Stevenson (1966) found that although there is a dramatic reduction in biochemical oxygen demand following chlorination, this reduction does not indicate total oxidation of organic molecules in the water. Chlorination may result in the synthesis of substituted chlorophenols or chlorophenolic compounds which are no longer susceptible to detection by measuring biochemical oxygen demand. Thus, chlorination may merely mask the presence of potentially hazardous organic molecules in drinking water.

D.7.4.4 Activated Carbon Adsorption

Activated carbon adsorption is an effective means of treating wastewater. Activated carbon has a strong affinity for nonpolar compounds such as phenols and would be expected to adsorb pentachlorophenol. The method is expensive and is not widely used, particularly in the treatment of wastewater from wood treatment plants. Data on the efficiency of pentachlorophenol adsorption by activated carbon was not found.

SECTION D.7

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D.8 ENVIRONMENTAL INTERACTIONS AND THEIR CONSEQUENCES

D.8.1 ENVIRONMENTAL CYCLING OF PENTACHLOROPHENOL

The sources, distribution, and fate of pentachlorophenol in soil, water, and air are discussed in Section D.7. No comprehensive investigations have been made on the movement of pentachlorophenol within or among these three segments of the environment. For example, it has been shown that pentachlorophenol volatilizes from aqueous solutions, but the rate and extent of volatilization from particular locations have not been studied. A possible environmental cycle for pentachlorophenol is presented in Figure A.8.1. Although the interactions shown have been verified (in most cases by laboratory studies), the extent of these processes in the environment is speculative. Further investigations--perhaps utilizing radiotracer techniques--are needed to clarify these interactions. Pentachlorophenol contamination of the environment may arise from three sources: (1) the manufacture and use of the compound as a wood preservative or pesticide, (2) degradation and resynthesis of other commonly used compounds, and (3) municipal chlorination of water containing phenolic compounds. The use of pentachlorophenol as a wood preservative is the most significant source of environmental pollution; the other two sources have not been investigated sufficiently. The data available, although scanty, provide some basis for speculation. This assessment may err on the side of caution since the hazards of low levels of pentachlorophenol in the environment have not been evaluated.

The effects of acute doses of pentachlorophenol on animals and humans are well documented. Acute intoxication can be easily avoided if appropriate safety measures are taken. Use of pentachlorophenol as a molluscicide, a herbicide, or an algicide should be carefully examined in light of the known toxic effects on aquatic and terrestrial wildlife. Sensitive species of fish perish at concentrations as low as 30 μg/liter (Section D.5.2.2.2.3).

The effects of chronic low levels of pentachlorophenol in humans and animals are unclear. Low levels in the urine of nonoccupationally exposed persons have been reported (Section D.6.2.2.4.2). Chronic exposure to pentachlorophenol was correlated with increased total bilirubin and creatine phosphokinase, gamma mobility, C-reactive protein, and liver enzyme activity. These findings, however, have not been corroborated and their clinical significance, if any, is unknown. In one investigation pentachlorophenol was detected in human adipose tissue (Section D.6.2.2.4.2). Accumulation in fatty tissue must be considered a distinct possibility, and the hazards associated with this accumulation warrant further investigation.

D.8.2 HUMAN EXPOSURE

Human exposure to pentachlorophenol may occur through air, water, and/or food. Exposure to pentachlorophenol in air has not been well documented, although acute pentachlorophenol poisoning by the respiratory route is known (Section D.6.1.1.1). Casarett et al. (1969) suggested that respiratory tract absorption could explain the almost ubiquitous presence of pentachlorophenol in the urine of nonoccupationally exposed persons. No reports assessed the relationship between airborne pentachlorophenol and concentrations of the compound in humans.

A similar lack of data prevents the adequate description of pentachlorophenol contamination of water. Pentachlorophenol levels in lake and river waters have been reported in the range of 0.01 to 10 mg/liter, but these values are based on a very few analyses and may not accurately reflect pentachlorophenol concentrations in the aquatic environment. Consequently, prevalent and ambient pentachlorophenol levels in water are rough estimates. The amount of human uptake of pentachlorophenol from water has not been determined.

More information is available on the levels of pentachlorophenol in foodstuffs. Since 1964, the U.S. Food and Drug Administration has conducted a program to monitor pesticide residues, including pentachlorophenol, in food. The sampling procedure involves gathering market baskets from 30 stores in 27 cities across the United States. Each basket consists of 82 to 117 food items and represents the dietary requirements of a male in the 16- to 19-year-old age group for a two-week period. The food items, chosen with the advice and assistance of the U.S. Department of Agriculture, are selected for that particular group because of the proportionally greater amount of food they consume. The contents of each sample basket are divided into 12 classes and aliquots are analyzed. Results are reported as either percentage of composite samples showing detectable pentachlorophenol levels and/or as average individual daily intake expressed as milligrams per person per day. From 1965 to 1969, 1% to 3.3% of the composite samples showed detectable levels of pentachlorophenol (Table D.6.36). Individuals consuming contaminated food were exposed to a daily dietary intake of pentachlorophenol ranging from 0.001 to 0.006 mg per person. Daily intake of pentachlorophenol from specific food categories is given in Table D.6.37. Pentachlorophenol does not appear to be present predominantly in any single food class.

Johnson and Manske (1976) surveyed the pesticide residues in market basket samples. They reported pentachlorophenol in 9 of 360 composites at levels of 0.01 to 0.02 mg/kg.

No information is available on specific geographical areas where contaminated food samples originated; therefore, it is impossible to correlate intake by humans with the extent of pentachlorophenol use or application. Although some portion of the U.S. population is exposed to low levels of pentachlorophenol through food consumption, it is not known whether the associated hazards are potential or actual.

D.8.3 BIOMAGNIFICATION IN FOOD CHAINS

The available data on environmental contamination by pentachlorophenol preclude definitive conclusions regarding the effects of pentachlorophenol in terrestrial ecosystems. Food chain biomagnification in terrestrial ecosystems has not been adequately studied and should be investigated. Pentachlorophenol biomagnification in aquatic systems is well documented, although the extent of the magnification and species variability have not been fully elucidated. Consequently, the effects of these processes on daily human intake of pentachlorophenol from food are not known. Investigations are needed so that government officials and the public can be fully appraised of the environmental impacts of pentachlorophenol and can determine if additional cautionary measures should be taken.

D.8.3.1 Terrestrial Ecosystems

The sources of pentachlorophenol contamination in foodstuffs have not been clearly described. Little information is available on distribution and transport in crops following pentachlorophenol application or exposure. In laboratory studies, pentachlorophenol was taken up by the root system of sugar cane grown in an aerated nutrient solution but was not translocated to other parts of the plant. However, pentachlorophenol or its metabolites may be translocated in cotton plants following spraying (Section D.4.1). Pentachlorophenol is not commonly applied directly to row crops intended for human consumption, but its ability to persist on foliage for at least eight weeks implies a potential hazard from spraying techniques. Pentachlorophenol application to pastureland has been reported (Section D.5.1.1.1.3), but this use is probably minimal. Cattle seem to avoid pentachlorophenol-treated grasses by preference of taste; however, the possibility exists that pentachlorophenol may be concentrated in the tissues of animals which consume the substance and that it eventually can be passed on to humans through the food chain.

Vermeer et al. (1974) reported extensive loss of wildlife following the application of sodium pentachlorophenate as a molluscicide in Surinam, South America (Section D.5.1.2.2.3). Snails, frogs, fish, and snail kites (a species of bird which feeds almost exclusively on snails) were found dead following pentachlorophenol application to rice fields. The kite mortality was attributed to consumption of snails which contained high concentrations of pentachlorophenol. This report is the only documentation of pentachlorophenol effects in a terrestrial food chain. Low, but detectable, pentachlorophenol levels were also found in other species of birds, but it is not clear whether these residue levels were due to consumption of other animals contaminated with pentachlorophenol or through direct contact with the compound. Further investigations are needed on the distribution of pentachlorophenol in plant and animal tissues, its movement through the food chain, and the airborne contributions from both agricultural applications and industrial sources.

D.8.3.2 Aquatic Ecosystems

Several studies on pentachlorophenol accumulation in aquatic organisms are available. In aquaria experiments, Kobayashi and Akitake (1975) exposed goldfish to 0.1 mg pentachlorophenol per liter for 120 hr (Section D.5.2.1.1). The fish concentrated the pentachlorophenol to a peak level of 90 mg/g of tissue, a concentration factor of 1000 over that of the original medium. Rudling (1970) analyzed pentachlorophenol levels in fish from a lake receiving pulp mill wastes. They found that pentachlorophenol levels in tissues ranged from 0.15 to 3.0 mg/g, representing concentration factors from 150 to 1000 over the amounts of pentachlorophenol in the lake water. Holmberg et al. (1972) found that pentachlorophenol accumulated in the tissues of eels kept in a medium containing 0.1 mg sodium pentachlorophenate per liter for eight days. Most of the stored pentachlorophenol was associated with lipids, and substantial amounts remained stored in the tissues after 55 days in pentachlorophenol-free water (Section D.5.2.1.2). The work of Lu and Metcalf (1975) is discussed extensively in Section D.5.2.2.2.6. In a closed laboratory system, these investigators traced the movement of pentachlorophenol through an aquatic food chain. The highest level of the chain was the mosquito fish, which accumulated and concentrated pentachlorophenol to a level 296 times that of the initial medium. Zitko, Hutzinger, and Choi (1974) detected levels of pentachlorophenol in aquatic organisms from a geographical area where the compound was not widely used. These studies indicate that accumulation and biomagnification of pentachlorophenol occur in the aquatic environment, and it is possible that a similar situation also exists for terrestrial systems.

SECTION D.8

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PART E

ENVIRONMENTAL ASSESSMENT OF CHLOROPHENOLS

PART E — ENVIRONMENTAL ASSESSMENT OF CHLOROPHENOLS

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E.1 INTRODUCTION

This environmental assessment of chlorophenols follows the organizational format of Parts A-D of this document. The discussion is divided into three groups of chlorophenol compounds: (1) monochlorophenols and dichlorophenols, (2) trichlorophenols and tetrachlorophenols, and (3) pentachlorophenol. Monochlorophenols and dichlorophenols are primarily used as intermediates in the synthesis of other products. Trichlorophenols are used directly as antimicrobial agents as well as intermediates in the synthesis of other chemicals. Tetrachlorophenols and pentachlorophenol are used extensively as fungicides for control of sap stain and for wood preservation. Evaluation of the environmental impact of the many products containing chlorophenol moieties, some of which are used extensively, is beyond the scope of this document. Additionally, the chemical processes used to produce chlorophenols also form a number of other contaminants, including other chlorophenols, hexachlorobenzene, chlorodibenzo-*p*-dioxins, chlorodibenzo-*p*-furans, and phenoxyphenols. The scientific evaluation of the environmental impact of these compounds is a separate task. A committee within the U.S. Environmental Protection Agency is presently reviewing the toxicology of chlorodioxins. This assessment focuses primarily on the toxicity and biological activity of chlorophenols.

E.2 MONOCHLOROPHENOLS AND DICHLOROPHENOLS

E.2.1 PRODUCTION, USES, AND POTENTIAL ENVIRONMENTAL CONTAMINATION

E.2.1.1 Production

2-Chlorophenol can be synthesized by diazotization of 2-chloroaniline, direct chlorination of phenol, or hydrolysis of 1,2-dichlorobenzene. The most common process is direct chlorination of phenol, yielding both 2- and 4-chlorophenol. 2-Chlorophenol is separated by fractional distillation. 2,4-Dichlorophenol is produced by direct chlorination of phenol or by further chlorination of 4-chlorophenol. Small amounts of 2,6-dichlorophenol and 2,4,5-trichlorophenol are also produced.

Production figures for 2-chlorophenol and 2,4-dichlorophenol are considered proprietary. Some indication of the volume produced can be obtained by looking at production of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Production of 2,4-D has been reported at various times to be from 24,000 to 38,000 metric tons per year.

reactions with formaldehyde produce phenolic resin intermediates or methylene bridged polymers. Under proper conditions the hydroxyl group may ionize, and in the presence of alkali metals metallic salts are formed. 2,4-Dichlorophenol also undergoes halogenation and nitration in the 6 position; further chlorination yields 2,4,6-trichlorophenol. Reactions with formaldehyde yield condensation products that are methylene bridged dimers. Salt formation is accomplished by reaction with alkali metals. The volatility of 2-chlorophenol and 2,4-dichlorophenol is likely a major dispersal mechanism of these chemicals in the atmosphere; however, monitoring data are lacking.

Although sorption of 2,4-dichlorophenol on clay minerals is insignificant (Aly and Faust, 1964), it can be sorbed to a large extent by ion-exchange resins (Aly and Faust, 1965). In general, strongly basic anion-exchange resins sorb more of the chemical than cation exchangers. Activated carbon has been used for removing low levels of chlorophenols and other organic compounds from water (Eichelberger, Dressman, and Longbottom, 1970).

Because chlorophenols may be found on surfaces where they are exposed to sunlight, they are susceptible to photochemical degradation. Aly and Faust (1964) demonstrated the rapid decomposition of an aqueous solution of a sodium salt of 2,4-D and 2,4-dichlorophenol exposed to ultraviolet light. Plimmer and Klingebiel (1971) studied the products of riboflavin-sensitized photodecomposition of 2,4-dichlorophenol in water irradiated at a wavelength of 280 nm. Major products identified were dimeric compounds; no dioxins were detected. Therefore, it seems unlikely that dioxins are formed from 2,4-dichlorophenol in aquatic systems.

E.2.2.2 Analytical Methods

The classic technique for analysis of chlorophenols in water is the 4-aminoantipyrine colorimetric method. This method is quite sensitive and easy to use, but it is nonspecific because the aminoantipyrine reacts with several isomers of chlorophenols to produce the same color.

Gas-liquid chromatography employing either electron capture or flame ionization is the most widely used technique for separating, identifying, and quantifying chlorophenols in various kinds of samples. This method is sensitive to nanogram levels and is adequate for the quantitation of chlorophenols. Biological samples such as animal and plant tissues require more extensive preparation than water samples before they are suitable for gas-liquid chromatographic analysis. Problems of poor resolution and tailing encountered in some samples can be corrected by judicious selection and preparation of column substrates (Kawahara, 1971; Karapally, Saha, and Lee, 1973; Shafik, Sullivan, and Enos, 1973; Baird et al., 1974). Because gas-liquid chromatography can detect nanogram quantities, contamination must be avoided by following proper techniques in sample collection, storage, and processing. Samples should be sealed and stored at low temperatures, preferably below freezing. Acidification with H_3PO_4 and $CuSO_4$ followed by refrigeration at 5°C to 10°C has been recommended for preservation of wastewater samples for phenol analysis (Rand, Greenberg, and Taras, 1976). Extraction and cleanup procedures for a variety of materials are available. The most common cleanup method is column chromatography.

E.2.2.3 Biological Degradation

Microbial decomposition appears to be the dominant mechanism for dissipation of chlorophenols in soils. Bacteria isolated from soils, including those known to degrade phenoxyacetic herbicides, are capable of metabolizing chlorophenols (Bollag, Helling, and Alexander, 1968; Evans et al., 1971; Spokes and Walker, 1974). Soil bacteria found to be active in 2,4-dichlorophenol decomposition include *Arthrobacter* sp. and *Pseudomonas* sp.

2-Chlorophenol disappears from soil with a half-life of less than 10 days following initial exposure and about twice as fast following subsequent exposures. 2,4-Dichlorophenol disappears completely from soil in 5 to 9 days; it disappears more rapidly than 2,4-D, which disappears in 30 days. The complete degradation involves ring cleavage and formation of succinic and, presumably, acetic acids. Although 2-chlorophenol and 2,4-dichlorophenol appear to be short-lived in soils, the data are incomplete; factors affecting their persistence need further study. Soil factors affecting sorption are pH, moisture, and the amount of clay and organic matter. Information on sorption of 2,4-dichlorophenol by organic matter is not available; therefore, its activity can only be inferred from studies on acidic pesticides such as 2,4-D. Acidic pesticide sorption to organic soil colloids is controlled by the pH of the system (i.e., sorption is greater under acidic conditions).

Fungi have been suspected to possess the ability to metabolize chlorophenols. Walker (1973) isolated a phenol-utilizing strain of *Rhodotorula glutinis* from a Rothamsted soil. It has not been determined if fungal strains are capable of utilizing chlorophenols as sole carbon sources and if degradation pathways of fungal metabolism are similar to those of bacterial metabolism.

Secondary treatment of sewage involves the removal of organic matter from wastewater by biological processes. Because 2-chlorophenol and 2,4-dichlorophenol are easily biodegradable, secondary treatment should provide excellent removal of these chemicals. Most of the chlorophenols are likely to decompose in the aeration lagoon. Optimum temperature for degradation of 2-chlorophenol in activated sludge appears to be between 25°C and 27°C, and optimum pH ranges from 6.5 to 8.0. Degradation was uninhibited at concentrations of 2,4-dichlorophenol up to 100 mg/liter (Ingols, Gaffney, and Stevenson, 1966). Neither 2-chlorophenol nor 2,4-dichlorophenol normally appear to pose a serious hazard in exposed waste treatment facilities; ready degradation by microbial populations in activated sludge and aeration lagoon effluent has been demonstrated. 2-Chlorophenol may persist longer due to direct or indirect toxic effects if wastes containing high levels are discharged into an unacclimated body of water.

E.2.3 EFFECTS ON LOWER LIFE FORMS

E.2.3.1 Effects on Bacteria, Fungi, and Algae

Both 2-chlorophenol and 2,4-dichlorophenol are bactericidal, fungicidal, and algicidal agents. In general, bactericidal and algicidal potency appears to increase with increased chlorine substitution of the parent phenol up to the trichlorophenol isomers. 2-Chlorophenol starts to have an effect on the chlorophyll content of algae (blue-green algae, *Chlorella pyrenoidosa*) at 10 mg/liter. At 500 mg/liter chlorophyll synthesis is inhibited. 2,4-Dichlorophenol is more toxic; concentrations of 100 mg/liter inhibit chlorophyll synthesis.

2-Chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol are more potent antibacterial agents than phenol. A concentration of 25 mg 2,4-dichlorophenol per liter inhibits the growth of *Pseudomonas* sp. At high concentrations chlorophenols act as gross protoplasmic poisons. At lower concentrations specific enzyme systems are inhibited. Concentrations of 0.015% to 0.030% of 2-, 3-, or 4-monochlorophenol inhibit the growth of *Aspergillus niger*.

E.2.3.2 Effects on Plants

The phytotoxicity of 2-chlorophenol to vascular plants has not been described, nor is information available on its distribution and transport in vascular plants. The metabolism of chlorophenoxyacetic acids to chlorophenols occurs in many plant species.

2,4-Dichlorophenol inhibits root growth in flax seedlings, cell expansion in wheat coleoptiles, and the growth-stimulating effect of indoleacetic acid in wheat (Volynets and Pal'chenko, 1972). The LC$_{50}$ of 2,4-dichlorophenol for the aquatic plant *Lemna minor* is 40 mg/liter. Additional information on phytotoxic properties is not available.

Very little information is available on the translocation and distribution of 2,4-dichlorophenol in vascular plants. Both oats and soybean will absorb 2,4-dichlorophenol from nutrient solutions and soil (Isensee and Jones, 1971). 2,4-Dichlorophenol was not found in the mature oat seed, but small quantities were found in the soybean seed. Oat seedlings concentrated 2,4-dichlorophenol in amounts up to nine times the soil level, but soybean shoots contained about the same level as the soil. Plant tissues were evaluated on a dry-weight basis. 2,4-Dichlorophenol applied to leaves of soybeans is not translocated. More than 98% of the 2,4-dichlorophenol applied was lost, probably due to volatilization, within 72 hr of application. Although 2,4-D and 2,4-dichlorophenol residues were found in rice plants during the first month after herbicide application, rice grains contained neither herbicide nor metabolite (2,4-dichlorophenol) when the plants reached maturity.

2,4-Dichlorophenol affects root mitosis in the European broad bean (*Vicia faba*) (Amer and Ali, 1969). Roots grown in a solution containing 62.5 mg 2,4-dichlorophenol per liter showed a decreased mitotic index and an increased frequency of induced mitotic anomalies including cytomixis, stickiness, lagging chromosomes, formation of anaphase bridges, and fragmentation effects. Spraying *Vicia faba* for five days with 7 ml of a solution of 0.39 mg 2,4-dichlorophenol per liter caused mitotic abnormalities in pollen mother cells (Amer and Ali, 1974). Similar results were obtained when seeds were soaked in the same solution and planted and pollen mother cells were obtained from the resulting plant.

Muhling et al. (1960) demonstrated that concentrations of 2,4-dichlorophenol greater than 60 mg/liter inhibited spindle formation in root cells of pea seedlings (*Pisum sativum*). The resulting chromosome configurations were similar to those produced by the mitotic inhibitor colchicine.

Information on the biotransformation of 2,4-dichlorophenol by plants is scanty. There is some evidence of 2,4-dichlorophenol detoxication through glycoside conjugation.

There is no evidence that plants are exposed to significant levels of monochlorophenols or dichlorophenols except as a result of intentional herbicide use. Accidental exposures are likely to be rare and limited in scope. If groundwater or irrigation water were contaminated, then phytotoxicity data would be useful in decision making.

E.2.4 EFFECTS ON ANIMALS

E.2.4.1 Effects on Fish and Other Aquatic Organisms

Data on the toxicity of 2-chlorophenol to aquatic organisms are sparse. The 48-hr LC_{50} for bluegill (*Lepomis macrochirus*) is 8.1 mg/liter, and for rainbow trout (*Salmo gairdneri*) the 96-hr LC_{50} is 2.6 mg/liter. The 48-hr LC_{50} for *Daphnia magna* is 7.4 mg/liter. 2,4-Dichlorophenol at 16.3 mg/liter decreases cell division in sea urchin eggs (*Arbacia punctulata*). The 48-hr LC_{50} of 2,4-dichlorophenol in *Daphnia magna* is 2.6 mg/liter.

Accidental spills into water would be expected to result in fish kills. The primary environmental concern is adequate processing of chemical waste streams to remove chlorophenols.

E.2.4.2 Effects on Birds, Wildlife, and Domestic Animals

No information was found on the metabolism or toxicologic effects of 2-chlorophenol or 2,4-dichlorophenol in birds, wildlife, or domestic animals. Sheep and cattle fed 2,4-D at levels of 60 mg/kg for 28 days, held for 7 days, and then killed contained 0.07 to 1.06 ppm 2,4-dichlorophenol in the kidney and 0.15 to 0.31 ppm 2,4-dichlorophenol in the liver (Clark et al., 1975). Levels of 2,4-D were similar to levels of 2,4-dichlorophenol in the liver but were higher in the kidney. Levels of 2,4-dichlorophenol in fat were less than 0.05 ppm, which is the detection limit, thus indicating that 2,4-dichlorophenol is not stored to any extent in body fat. Bjerke et al. (1972) reported that 2,4-dichlorophenol does not occur in milk from cows fed high levels of 2,4-D.

2,4-Dichlorophenol was found in egg yolk at levels of 0.1 to 0.6 ppm after chickens were fed a diet containing 100 to 800 mg nemacide (*O*-(2,4-dichlorophenyl)*O*,*O*-diethyl phosphorothioate) per kilogram of feed for 55 weeks (Sherman, Beck, and Herrick, 1972). Residues of 0.3 to 0.5 ppm 2,4-dichlorophenol were also found in the liver but not in breast muscle of the hens. Biotransformation, elimination, kinetics, and toxicologic effects were not described.

E.2.4.3 Effects on Laboratory Animals

The oral LD_{50} for 2-chlorophenol in the rat, mouse, and fox ranges from 440 to 670 mg/kg. Clinical signs include restlessness, increased respiration, tremors, convulsions, motor weakness, and coma. Lesions include kidney damage, fatty liver, hemorrhages, and necrosis in the stomach and intestine. 2-Chlorophenol is a convulsant with seizures occurring 1 to 2 min after intraperitoneal injection. 2-Chlorophenol appears to be a weak uncoupler of oxidative phosphorylation. Dogs eliminate 2-chlorophenol in the urine primarily as sulfuric and glucuronic conjugates. The chronic toxicity has not been reported. A 90-day, multiple-level feeding study with rodents would be the first step in filling this data gap.

The oral LD_{50} for 2,4-dichlorophenol is 580 mg/kg in the rat and 1600 mg/kg in the mouse. 2,4-Dichlorophenol has been fed to mice for six months at levels of 100 or 230 mg/kg. The 100 mg/kg dose was considered to be a no-effect level. There is no evidence to suggest that 2,4-dichlorophenol is a convulsant.

The metabolites or biotransformation products of 2,4-dichlorophenol have not been reported for humans or experimental animals. The only information on the metabolism of 2-chlorophenol and 2,4-dichlorophenol is derived from studies on chlorobenzene, lindane, and nemacide where 2-chlorophenol and 2,4-dichlorophenol occur as degradation products. Studies on 1,2,3,4,5,6-hexachlorocyclohexane in mice have shown the elimination of sulfate and glucuronide conjugates of 2,4-dichlorophenol in the urine. 2,4-Dichlorophenol is a metabolite of 1,2,3,4,5,6-hexachlorocyclohexane. The results indicate that 2,4-dichlorophenol is rapidly eliminated.

E.2.5 EFFECTS ON HUMANS

There is no direct information on the toxicity of 2-chlorophenol or 2,4-dichlorophenol in humans. The individual most likely to be exposed is the industrial worker, except for exposure of the general population to minute quantities of these chlorophenols which occur in the environment as degradation products of other compounds.

E.2.4.6 TERATOGENICITY, MUTAGENICITY, AND CARCINOGENICITY

Mutagenic and teratogenic effects have not been studied. 2-Chlorophenol and 2,4-dichlorophenol have skin tumor-promoting activity similar to that of phenol when tested in mice following a single initiating dose of dimethylbenzanthracene. The lack of information on the carcinogenicity of these chlorophenols is considered to be the most significant data gap for monochlorophenols and dichlorophenols.

E.2.7 RESIDUES IN FOOD AND WATER

Data indicate that contamination of food with 2-chlorophenol or 2,4-dichlorophenol is minimal, although 2,4-dichlorophenol has occasionally been reported as a food contaminant. 2,4-Dichlorophenol has been reported in potato tubers (Bristol et al., 1974). The levels reported are low, infrequently found, and are not cause for concern.

Dichlorophenols have been implicated as compounds contributing to the objectionable phenolic odor and taste of water. The threshold odor and taste concentration for 2,4-dichlorophenol is 2 to 8 μg/liter (2 to 8 ppb). The formation of chlorophenols during the chlorination of drinking water has been reported (Burttschell et al., 1959). 2-Chlorophenol can be converted to 2,4-dichlorophenol and 2,6-dichlorophenol; 2,4-dichlorophenol can proceed further to form 2,4-6-trichlorophenol.

E.3 TRICHLOROPHENOLS AND TETRACHLOROPHENOLS

E.3.1 PRODUCTION, USES, AND POTENTIAL ENVIRONMENTAL CONTAMINATION

E.3.1.1 Production

There are two trichlorophenol isomers and one tetrachlorophenol isomer of commercial interest: 2,4,6- and 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol. Other tetrachlorophenol isomers are 2,3,4,5- and 2,3,5,6-tetrachlorophenol. 2,4,6-Trichlorophenol is formed by the direct chlorination of phenol. 2,4,5-Trichlorophenol is produced through the hydrolysis of 1,2,4,5-tetrachlorobenzene. Both isomers can also be produced by diazotization of the corresponding trichloroaniline isomer. 2,3,4,6-Tetrachlorophenol and 2,3,4,5-tetrachlorophenol can be produced by direct chlorination of phenol or by further chlorination of lower chlorophenols. 2,3,4,5-Tetrachlorophenol can also be formed by diazotization of 2,3,4,5-tetrachloroaniline or hydrolysis of pentachlorobenzene. 2,3,5,6-Tetrachlorophenol can be synthesized from 2,3,5,6-tetrachloroaniline or as a coproduct with 2,3,4,5-tetrachlorophenol in the hydrolysis of pentachlorobenzene.

Production figures for trichlorophenols and tetrachlorophenols have not been reported in recent years. The fact that large amounts are probably produced can be inferred from production figures from 1965 to 1968, when from 1,817 to 12,742 metric tons of 2,4,5-trichlorophenol was produced each year. Because data from recent years have not been made available, trends in production cannot be assessed.

E.3.1.2 Uses

2,4,6-Trichlorophenol is used as a bactericide and fungicide in the preservation of wood, leather, and glue and in the treatment of mildew on textiles. It is also used as an ingredient in the preparation of insecticides and soap germicides. The primary use of 2,4,5-trichlorophenol is as an intermediate in the synthesis of pesticides such as the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or the insecticide ronnel. 2,4,5-Trichlorophenol is also used as a germicide and as an ingredient in soap germicides. 2,3,4,6-Tetrachlorophenol is used as a preservative for wood, latex, and leather and also as an insecticide. 2,3,4,5-Tetrachlorophenol and 2,3,5,6-tetrachlorophenol have not been used commercially.

E.3.1.3 Environmental Residues

Data are lacking on the presence of trichlorophenols and tetrachlorophenols in the environment. The presence of microbiological degradation mechanisms may mean that residues do not exist to any appreciable extent. Another possibility is that general environmental contamination has not been evaluated. Data on levels of trichlorophenols and tetrachlorophenols in the air are not available.

Trichlorophenols disappear in a few days from aerated lagoons or activated sludge systems; therefore, it is unlikely that a significant contamination problem would occur as long as wastewaters are treated (Ingols, Gaffney, and Stevenson, 1966; Sidwell, 1971). Trichlorophenols at levels of 2 to 20 mg/liter have been reported in wastewater from an industrial plant manufacturing herbicides (Sidwell, 1971). The contribution of runoff to chlorophenols in water following herbicide use has not been evaluated.

Trichlorophenols enter the environment through their use as fungicides or as degradation products of the herbicides 2,4,5-T and silvex [2-(2,4,5-trichlorophenoxy)propionic acid]. Tetrachlorophenols enter the environment as a result of their use as fungicides to preserve wood. Also, commercial pentachlorophenol, which is used extensively as a wood preservative, contains 4% to 10% tetrachlorophenol.

Many commonly used organochlorine compounds are degraded by mammals to yield higher chlorophenols. Compounds that result in trichlorophenol degradation products include 2,4,5-T; silvex; alpha, beta, gamma, and delta isomers of hexachlorocyclohexane; ronnel; pentachlorobenzene; and trichlorobenzene. Compounds yielding tetrachlorophenol degradation products include γ-hexachlorocyclohexane (lindane), tetrachlorobenzene, pentachlorobenzene, and pentachlorophenol. Exposure to one of these compounds can result in trace residues of chlorophenols in animals or humans. For example, Johnson and Manske (1976) reported on pesticide residues in 360 food composites found in a Food and Drug Administration market basket survey from 1972 to 1973. Pesticides which degrade to chlorophenols were found as follows: pentachlorophenol in 9 composites at levels of 10 to 20 ppb, lindane in 39 composites at levels of 0.3 to 6.0 ppb, ronnel in

2 composites at trace levels, and 1,2,3,4,5,6-hexachlorocyclohexane in 59 composites at levels of 0.2 to 5.0 ppb. If these low-level residues are degraded in humans to lower chlorophenols, the resulting levels will be even lower and, based on present evidence, are of no identifiable health concern.

E.3.2 ENVIRONMENTAL PERSISTENCE

E.3.2.1 Chemical and Physical Properties

The molecular weight of trichlorophenols is 197.46. 2,4,6-Trichlorophenol melts at 68°C, boils at 246°C, and has a vapor pressure of 1 mm Hg at 76.5°C. 2,4,5-Trichlorophenol melts at 66°C and sublimes at 245°C; its vapor pressure is 1 mm Hg at 72°C. Trichlorophenols are slightly soluble in water and very soluble in organic solvents.

Water-soluble salts of 2,4,6- and 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol are readily prepared. Trichlorophenols and tetrachlorophenols are weak acids. It has been shown that 2,4,5-T will photodecompose to form 2,4,5-trichlorophenol, which, in turn, forms a colored polymeric product (Crosby and Wong, 1973). Leopold, van Schaik, and Neal (1960) reported charcoal absorption properties for 18 chlorinated phenoxyacetic acids. One, two, or three chlorine substitutions increased absorptive properties.

E.3.2.2 Analytical Methods

Sample extraction and cleanup methods are available for a variety of sample matrices. Gas-liquid chromatography, the most widely used technique for separating, identifying, and quantifying chlorophenols in various kinds of samples, is sensitive at nanogram levels. Either electron-capture or flame-ionization detectors are used. Other methods include colorimetry, thin-layer chromatography, ultraviolet and infrared spectrophotometry, and mass spectrometry. Problems of poor resolution and tailing can be corrected by judicious selection of columns and type of derivatization. With the known toxicity of the trichlorophenols and tetrachlorophenols, present analytical methods are adequate.

E.3.2.3 Biological Degradation

Microbial degradation appears to be a major elimination mechanism. Bacterial species capable of metabolizing 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol have been isolated from soil and activated sludge (Tabak, Chambers, and Kabler, 1964; Nachtigall and Butler, 1974). The metabolic pathways have not been outlined, and the extent to which such bacteria are present in the environment is not known. Under experimental conditions, 2,4,6-trichlorophenol at concentrations of 50 to 100 ppm inhibits oxygen uptake in mixed microbial populations but has no effect at 1 to 10 ppm. The importance of this effect on sewage treatment processes is unknown.

Three isomers of tetrachlorophenol (2,3,4,5-, 2,3,5,6-, and 2,3,4,6-) have been shown to disappear from soil to a large degree in four weeks (Ide et al., 1972). Several genera of bacteria are capable of metabolizing chlorophenols (Chambers, Tabak, and Kabler, 1963; Tabak, Chambers, and Kabler, 1964). 2,4,5-Trichlorophenol is more resistant to soil microbial degradation than is 2,4,6-trichlorophenol, and 2,4,5-T is more resistant than 2,4,5-trichlorophenol. One major source of 2,4,5-trichlorophenol in soil is as a breakdown product of 2,4,5-T; as long as 2,4,5-T is not present, it is unlikely that 2,4,5-trichlorophenol will be present as a result of using 2,4,5-T.

Kearney, Woolson, and Ellington (1972) incubated soil with 2,4,5-trichlorophenol and reported no evidence of microbial condensation to chlorodioxins. Sidwell (1971) reported that wastewater treatment by a combined lagoon and stabilization pond removed 87% to 94% of the chlorophenols. Soil sorption characteristics of trichlorophenols and tetrachlorophenols have not been studied.

E.3.3 EFFECTS ON LOWER LIFE FORMS

E.3.3.1 Effects on Bacteria, Fungi, and Algae

2,4,6-Trichlorophenol is about 24 times more effective as a bactericide than is phenol. Some research has demonstrated that 2,4,6-trichlorophenol affects bacterial membrane permeability. Similar studies with 2,4,5-trichlorophenol have not been reported.

2,4,5-Trichlorophenol inhibits growth of bacteria at concentrations of 10 to 400 mg/liter. The test organism, *Pseudomonas aeruginosa*, was more resistant than other organisms tested. Bacteria capable of metabolizing 2,4,6-trichlorophenol have been isolated from soil and activated sludge. 2,4,5-Trichlorophenol inhibits a number of fungal species at concentrations of 2 to 5 mg/liter (ppm). Most fungi are inhibited at concentrations around 10 ppm.

In one study, 99 of 116 fungi isolated from poultry litter metabolized tetrachlorophenols, as evidenced by a loss of tetrachlorophenols from the culture (Gee and Peel, 1974). Twenty-two of the 26 fungal species metabolized tetrachlorophenols at rates ranging from 4% to 100% in five days. Some fungi both metabolized tetrachlorophenols and formed chloroanisoles. Bacteria isolated from poultry litter have been shown to completely metabolize 2,3,4,6-tetrachlorophenol in five days without the production of chloroanisole. Aerobic conditions were required.

2,4,5-Trichlorophenol and 2,4,6-trichlorophenol inhibit chlorophyll synthesis in *Chlorella pyrenoidosa* (blue-green algae) at concentrations greater than 2.5 mg/liter (2.5 ppm). Concentrations of 1 mg/liter have only a minimal effect. These compounds pose a threat to algae in waste stabilization ponds.

E.3.3.2 Effects on Plants

Information on the effects, biotransformation, and elimination of trichlorophenols and tetrachlorophenols in plants is not available. No dose-response data have been reported for vascular plants. Blackman, Parke, and Garton (1955) reported the following LC_{50} values for the aquatic plant *Lemna minor*: 0.6 mg/liter 2,3,4,5-tetrachlorophenol, 1.6 mg/liter 2,4,5-trichlorophenol, and 5.9 mg/liter 2,4,6-trichlorophenol. In another study, 2,4,6-trichlorophenol had no effect on plants when tested for eight months at concentrations of 0.01 to 4.1 mg/liter (Manufacturing Chemists Association, 1972). The data gap in this area would be important if irrigation water became contaminated.

E.3.4 EFFECTS ON ANIMALS

E.3.4.1 Effects on Aquatic Organisms

No information is available on the toxicity of 2,4,5-trichlorophenol to aquatic organisms. The 96-hr median tolerance limit of 2,4,6-trichlorophenol for the fathead minnow is 0.6 mg/liter. Other reliable data are not available. Acute aquatic toxicity studies are needed to fill this data gap.

E.3.4.2 Effects on Birds

The fungal metabolism of chlorophenols to chloroanisoles in chicken litter has caused an economic problem in England. The meat and eggs acquire a musty taint. There is no expected health hazard because the meat and eggs are unpalatable. This problem can largely be avoided by not using wood shavings treated with sap-stain control agents containing chlorophenols (Curtis et al., 1974).

E.3.4.3 Effects on Mammals

E.3.4.3.1 General Considerations — 2,4,5-Trichlorophenol and 2,4,6-trichlorophenol are absorbed from the gastrointestinal tract. Skin contact can result in erythema, edema, and chemical burning. Apparently, 2,4,5- and 2,4,6-trichlorophenol do not penetrate intact skin in sufficient amounts to result in systemic toxicity. 2,3,4,6-Tetrachlorophenol dissolved in organic solvents can be absorbed through intact skin in toxic amounts. Data on the rate of uptake of trichlorophenols and tetrachlorophenols are not available.

E.3.4.3.2 Effects on Domestic Animals — No toxic effects were reported in cattle fed 2,4,5-trichlorophenol at levels of 18 or 159 mg/kg for 78 days and 53 mg/kg for 154 days (Anderson et al., 1949). Feed consumption and weight gain were unaffected.

Because 2,4,5-trichlorophenol is a metabolite of 2,4,5-T, additional insight on the toxicity of 2,4,5-trichlorophenol can be obtained from studies in which 2,4,5-T was fed to animals. Cattle were fed 2,4,5-T at levels from 10 to 1000 mg/kg feed (Bjerke et al., 1972). Lactating cows fed 1000 mg 2,4,5-T per kilogram of feed for 21 days eliminated 0.2 to 1.0 ppm 2,4,5-T and 0.15 to 0.25 ppm 2,4,5-trichlorophenol in milk. Levels in milk decreased to less than 0.05 ppm for both 2,4,5-T and 2,4,5-trichlorophenol within 3 days after feeding was stopped. At feeding levels of 100 mg/kg, 2,4,5-T did not appear in milk and 2,4,5-trichlorophenol was present at 0.05 to 0.07 ppm. Based on available data, there should be no significant residue problem with low-level exposure of cattle to these compounds.

Sheep were fed 2,4,5-T for 28 days at the rate of 60 mg/kg (Clark et al., 1975). 2,4,5-Trichlorophenol residues were found in muscle (0.13 ppm), liver (6.1 ppm), and kidney (0.9 ppm) but not in fat. Tissue residues of 2,4,5-T decreased markedly during a 7-day withdrawal period, but 2,4,5-trichlorophenol levels did not decrease as rapidly. Additional information on the kinetics and biotransformation of these compounds in domestic animals is not available.

E.3.4.3.3 Effects on Laboratory Animals — In the rat the oral LD_{50} for 2,4,5-trichlorophenol ranges from 820 to 2900 mg/kg and for 2,4,6-trichlorophenol from 820 to 2800 mg/kg. In the rat and mouse the LD_{50} for 2,3,4,6-tetrachlorophenol administered by the oral, subcutaneous, or intraperitoneal route ranges from 120 to 250 mg/kg. 2,4,6-Trichlorophenol has convulsant properties. 2,4,5-Trichlorophenol and 2,3,4,6-tetrachlorophenol do not cause convulsions but result in hypotonia. High doses of 2,4,5-trichlorophenol cause diarrhea. Feeding rabbits 20 doses of 1 or 10 mg 2,4,5-trichlorophenol per kilogram over 28 days did not result in toxicosis (McCollister, Lockwood, and Rowe, 1961). Doses as high as 500 mg/kg resulted in only slight kidney and liver changes. Rats fed 10, 30, or 100 mg/kg did not know toxicologic changes. Dosages of 300 or 1000 mg/kg resulted in minor histopathologic changes in kidney and liver. The mouse excretes 2,4,6-trichlorophenol in the urine as sulfate and glucuronide conjugates. The kinetics for elimination have not been described.

Trichlorophenols inhibit oxidative phosphorylation but to a lesser degree than tetrachlorophenols and pentachlorophenol. Tetrachlorophenols, like pentachlorophenol, causes hyperpyrexia. Trichlorophenols cause only small elevations in body temperature. Death from trichlorophenols and tetrachlorophenols results in rapid onset of rigor mortis, which also has been reported with pentachlorophenol.

E.3.5 EFFECTS ON HUMANS

Human systemic poisoning with 2,4,5- and 2,4,6-trichlorophenol and tetrachlorophenols has not been reported. The industrial worker is most likely to be exposed to trichlorophenols. Information on general population exposure is unavailable. The lower chlorophenols have not been reported in food, but they may be present in water as a result of chlorination.

The manufacturing process for trichlorophenols or products manufactured from trichlorophenol intermediates has resulted in the formation of the highly toxic contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is a potent chloroacnegenic agent. Chloracne has occurred in industrial plants manufacturing trichlorophenols. Improved manufacturing processes have resulted in much lower 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in trichlorophenol products. The commercial process used and the methods for destroying dioxins are patented. The toxicology of dioxins is beyond the scope of this assessment of chlorophenols and is an issue that should be considered separately.

E.3.6 CARCINOGENICITY AND TERATOGENICITY

Innes et al. (1969) reported that the oral administration of 2,4,6-trichlorophenol to mice yielded ambiguous results; additional studies are needed. 2,4,6-Trichlorophenol applied to the skin of mice along with an initiating dose of dimethylbenzanthracene did not produce tumors (Boutwell and Bosch, 1959). 2,4,5-Trichlorophenol with dimethylbenzanthracene produced tumors, but 2,4,5-trichlorophenol alone did not. Additional research is needed to fill this data gap.

2,4,5-Trichlorophenol was not teratogenic in mice or rats at doses of 0.9 or 9.0 mg/kg (Neubert and Dillmann, 1972). No information on the teratogenicity of 2,4,6-trichlorophenol or tetrachlorophenols was found; research is needed.

E.3.7 STANDARDS AND REGULATIONS

No standards or regulations dealing with trichlorophenols or tetrachlorophenols were found.

E.4 PENTACHLOROPHENOL

E.4.1 PRODUCTION, USES, AND POTENTIAL ENVIRONMENTAL CONTAMINATION

E.4.1.1 Production

Pentachlorophenol has been produced in significant quantities since the 1930s. Production in 1972 was 23 million kilograms; current production is an estimated 25 million kilograms. Pentachlorophenol is formed by the chlorination of phenol. Catalysts used include iron, aluminum, or antimony chlorides. Presently, pentachlorophenol is produced at four locations in the United States; very little is imported. One U.S. producer has indicated an intent to discontinue manufacture.

E.4.1.2 Uses

About 90% to 95% of the pentachlorophenol used in the United States is for the preservation of wood. Technical grade pentachlorophenol typically contains 4% to 10% tetrachlorophenol. In 1975, 244 million cubic feet of wood were treated with some type of preservative. Of this amount, 60 million cubic feet were treated with pentachlorophenol. The greatest uses of pentachlorophenol-treated wood are for poles, lumber, and fence posts.

Pentachlorophenol is used in either the water-insoluble phenol form or as the water-soluble sodium salt, sodium pentachlorophenate. Pentachlorophenol is used as a herbicide, a preharvest desiccant, an algicide, a molluscicide, and for slime and mold control in food processing plants and pulp mills. It is also used as a fungicide in the processing of cellulosic products, starches, adhesives, paints, leathers, oils, rubber, textiles, and wooden crates used for packaging raw agricultural products.

E.4.1.3 Losses to the Environment

Because the primary use of pentachlorophenol is in the preservation of wood, pentachlorophenol is intentionally applied to focal areas in the environment. Its use as a herbicide, except for small quantities in spot treatments, appears to be limited at this time. Its use as a molluscicide is also limited. The continued use of pentachlorophenol as a general herbicide or molluscicide should be reconsidered in the context of current practices and alternatives.

There are no reliable data on pentachlorophenol emissions from manufacturing plants. Ifeadi (1975) estimated losses in the manufacturing process to range from 0.55 to 2.16 kg/metric ton. Losses to the environment at the manufacturing site need to be quantified.

Pentachlorophenol also enters the environment from the several hundred wood treating plants in North America. The principal route is surface drainage into waterways. There is also some atmospheric loss due to vaporization, but these losses have not been quantitated. An indicator of significant losses would be damage to green plants in the area or fish kills. Pentachlorophenol has been found in water draining from wood treating plants (Thompson and Dust, 1971; Bevenue et al., 1972).

Treated wood can serve as a point source. Studies have shown that pentachlorophenol-oil solutions migrate down the vertical axes of poles, and some solution leaves the wood at ground level. Contamination is limited to the few inches of soil immediately adjacent to the pole. Losses are highest from freshly treated wood, but such losses are usually of no consequence. Occasionally, sensitive ornamental plants are damaged when freshly treated poles or lumber are used immediately adjacent to them. Under field conditions, grasses or other plants are usually seen growing around the pole or fence post.

E.4.1.4 Environmental Residues

Pentachlorophenol is present at low levels in the environment. It was found in the urine of nonoccupationally exposed persons at a level of 0.01 to 0.2 ppm (Casarett et al., 1969). One study found an average level of 26.3 ppb in human fat (Shafik, 1973). Levels in the range of 0.05 to 0.3 ppm in the blood plasma of nonoccupationally exposed persons have also been reported (Casarett et al., 1969).

Bevenue, Ogata, and Hylin (1972) reported 2 to 284 ng pentachlorophenol per liter (2 to 284 ppt) in rainwater and 14 ppt in snow. Pentachlorophenol has been reported in drinking water at levels of 0.1 to 0.3 ppb (0.1 to 0.3 µg/liter) (Buhler, Rasmusson, and Nakaue, 1973; Arsenault, 1976). Buhler, Rasmusson, and Nakaue (1973) reported pentachlorophenol levels of 0.1 to 0.7 ppb in river water, and levels of 0.01 to 10 ppb were reported by Goto (1971) in rivers of Japan.

Zitko, Hutzinger, and Choi (1974) reported pentachlorophenol residues of 1 to 10 ppb in fish and bird eggs obtained from a marsh or aquatic environment. Pentachlorophenol residues of 150 to 200 ppb were reported by Rudling (1970) in fish from a lake receiving pulp mill wastes. Levels in eels were 3000 ppb. Fish have been shown to accumulate and concentrate pentachlorophenol by factors of 150 to 1000 (Rudling, 1970; Holmberg et al., 1972; Lu and Metcalf, 1975; Glickman et al., 1977; and Pruitt, Grantham, and Pierce, 1977). There appear to be species differences in the ability of fish to concentrate pentachlorophenol.

Pentachlorophenol was found in the Food and Drug Administration market basket survey samples at a frequency of 1.4% to 3.3% (Duggan and Corneliussen, 1972). The resulting human intake was estimated to be 1 to 6 µg per person per day.

From the above information, it is apparent that parts-per-trillion to parts-per-billion levels of pentachlorophenol are present in the environment. Clearly, the manufacture and use of pentachlorophenol contribute to these residues; however, there are other potential sources. Arsenault (1976) reported that 10 ppm chlorine can chlorinate 1 ppm of naturally occurring phenol, yielding approximately 0.2 ppb pentachlorophenol. Considerable evidence shows that chlorination results in the formation of lower chlorinated phenols, but the report by Arsenault (1976) is the only one suggesting that pentachlorophenol may be generated by municipal chlorination. This experiment needs to be replicated. Evidence also exists that pentachlorophenol may be a degradation product of other chemicals, including hexachlorobenzene and lindane (Lui and Sweeney, 1975; Mehendale, Fields, and Matthews, 1975; Engst, Macholz, and Kujawa, 1976; Engst et al., 1976; Kohli et al., 1976). The quantitation of these degradation pathways and the contribution of these metabolic products to overall pentachlorophenol residues require further study.

E.4.2 ENVIRONMENTAL PERSISTENCE

E.4.2.1 Physical and Chemical Properties

In the pure state pentachlorophenol is a white, crystalline solid with a molecular weight of 266.35 and a vapor pressure at 20°C of 0.00011 mm Hg. Its water solubility is 14 ppm (14 mg/liter) at 20°C. It is soluble in alcohols, ethers, and various organic solvents, and is commonly dissolved in aromatic hydrocarbons, heavy oils, or light solvents such as mineral spirits when it is used to treat wood. The melting point is 190°C and the boiling point is 293°C.

A monovalent alkali metal salt of pentachlorophenol which is water soluble can be formed; most commonly this salt is sodium pentachlorophenate. Pentachlorophenol is quite stable. It does not decompose when heated at temperatures up to its boiling point (293°C) for extended periods. Pure pentachlorophenol is considered to be rather inert chemically. At pH 5 or lower, pentachlorophenol is nonionized.

Pentachlorophenol is lost from treated wood slowly. Early losses from wood can result from migration of the carrier solvent. Treated wood retains pentachlorophenol for years, as evidenced by the preservation of wood for 30 years or more. The rate of pentachlorophenol loss varies with the solvent system. Pentachlorophenol mixed with soil and placed in a glass jar with only a paper cover persisted for 5 years (Hetrick, 1952).

Pentachlorophenol disappears rapidly from aerated solutions, presumably by vaporization. Pentachlorophenol undergoes photodecomposition and forms a variety of products (Munakata and Kuwahara, 1969). The degradation products show stronger fungicidal properties but weaker phytotoxic and fish-killing activities than pentachlorophenol.

E.4.2.2 Analytical Methods

The most widely used, effective technique for analysis of pentachlorophenol in many sample types is electron-capture gas-liquid chromatography. Confirmation techniques include ultraviolet and infrared spectrophotometry as well as mass spectroscopy. Current detection limits of nanogram to picogram quantities are sufficient in view of the toxicity of the compound.

E.4.2.3 Biological Degradation

Pentachlorophenol is an organic molecule and its ultimate fate under aerobic conditions should be degradation to chlorine, carbon dioxide, and water. Bacterial metabolism and detoxification of pentachlorophenol occurs in the environment and in the laboratory. Certain soil bacteria detoxify pentachlorophenol by methylation to form pentachloroanisole (Cserjesi, 1972). Chu and Kirsch (1972, 1973) isolated a bacterial strain from a continuous-flow enrichment culture which metabolized pentachlorophenol as a sole source of organic carbon and energy. Others have cultured bacteria that biodegrade significant quantities of pentachlorophenol (Kirsch and Etzel, 1973; Watanabe, 1973). Adding pentachlorophenol to soil results in an increase in the number of pentachlorophenol-decomposing microorganisms (Watanabe, 1973). Suzuki (1977) reported that *Pseudomonas* sp. (bacteria) released $^{14}CO_2$ when [^{14}C]pentachlorophenol was added to cultures. Other metabolites included tetrachlorocatechol and tetrachlorohydroquinone. Certain fungi have also been shown to methylate pentachlorophenol to form pentachloroanisole (Curtis et al., 1974).

Mobility and persistence in soils depends on the physical and chemical characteristics of the soil as well as on the microbiological population. Soil factors include temperature, moisture, soil type, organic matter content, free iron oxides, clay minerals, sorptivity, and cation-exchange capacity. The interrelationships among these factors are complex. Adequate soil moisture increases pentachlorophenol degradation; pentachlorophenol is relatively stable in dry soils. Persistence is longer in heavy-clay soils and in soils with low organic content. Sterilizing soil samples has been shown to stop the degradation of pentachlorophenol. Under what might be considered natural field conditions, there is 90% degradation in 30 to 50 days.

Sidwell (1971) demonstrated that less than 1% of added pentachlorophenol was present after 30 hr in an aerated lagoon secondary wastewater treatment system. Other experiments have been performed with activated sludge treatment of wastewater from wood preserving plants. The system degrades pentachlorophenol, but shock loading of municipal plants is a potential problem. Each situation must be considered separately. Evidence suggests that pentachlorophenol-containing wastewaters can be successfully treated.

E.4.3 EFFECTS ON LOWER LIFE FORMS

E.4.3.1 Effects on Bacteria, Fungi, and Algae

Pentachlorophenol and sodium pentachlorophenate are well-known, widely used antimicrobial substances. Inhibition of bacterial and fungal growth occurs at concentrations from less than 1 ppm up to 2500 ppm (mg/liter). Typical inhibitory concentrations range from 10 to 80 ppm. Some fungi show tolerance to pentachlorophenol concentrations up to 100 ppm. Fortunately, these fungi do not produce wood decay. Pentachlorophenol is toxic to algae. Levels of 2 to 20 ppm inhibit growth.

E.4.3.2 Effects on Plants

Pentachlorophenol has been used as a herbicide. Small amounts of pentachlorophenol applied to leaves will migrate to the stalk and to other leaves in sugar cane. Sugar cane will also take up pentachlorophenol from a nutrient media. Most (99%) of the pentachlorophenol was in the roots with the remainder in the stalk and suckers. There is some evidence that sugar cane roots metabolize pentachlorophenol (Hilton, Yuen, and Nomura, 1970). Pentachlorophenol is also translocated in cotton plants (Miller and Aboul-Ela, 1969).

Pentachlorophenol is toxic to plants, but there are species differences in susceptibility. In the past, greenhouse flats have been treated with pentachlorophenol, but this practice is generally not recommended. Pentachlorophenol-treated wooden posts or stakes have been used to support plants in orchards. Adverse effects result if the stakes are freshly treated or if the wood is placed too close to the plant. Pentachlorophenol is also toxic to aquatic plants.

E.4.4 EFFECTS ON ANIMALS

E.4.4.1 Effects on Fish

Fish kills have occurred when sufficiently high levels of pentachlorophenol effluents have entered waterways. There is considerable variation in the susceptibility of different aquatic organisms, with levels ranging from 20 to 9500 ppb. In general, the 96-hr LC_{50} values are on the order of 32 to 340 ppb for salmon, trout, bluegill, and fathead minnows. Some of the variation in reported values results from different experimental methodologies. The chronic toxicity of pentachlorophenol to fish has not been determined. Whole-body tissue residues in fish killed by pentachlorophenol ranged from 30 to 60 ppm (Vermeer et al., 1974).

Biomagnification of pentachlorophenol has not been demonstrated in the environment but has been shown in the laboratory by Lu and Metcalf (1975). The highest trophic level was the mosquito fish. The ecologic magnification factor for pentachlorophenol was 296 versus 16,950 for DDT, 1312 for aldrin, and 29 for nitrobenzene. The rapid elimination of pentachlorophenol from most organisms probably contributes to the relatively low magnification even though pentachlorophenol is rapidly absorbed. Lu and Metcalf (1975) reported that pentachlorophenol was a degradation product of hexachlorobenzene in their model aquatic ecosystem.

Several studies have demonstrated that fish rapidly take up pentachlorophenol from the water. Fish have the ability to accumulate pentachlorophenol to levels 100 to 1000 times the ambient water level. Highest levels are found in blood, gills, hepatopancreas, gallbladder, kidney, heart, and skin. Muscle has lower levels than do most other tissues. Contaminated fish placed in clean water rapidly eliminate a major portion of the residue. Most studies have been for short durations, but some evidence suggests a longer retention of lower-level residues. Present data are inadequate to define the long-term toxicokinetics in fish, and additional research is needed. Future studies should consider effects of the water pH, which has a significant effect on ionization of pentachlorophenol.

E.4.4.2 Effects on Birds

Few studies on the toxicity of pentachlorophenol to birds have been reported. In one field investigation, dead birds were found in a rice field treated with sodium pentachlorophenate (Vermeer et al., 1974). Analysis of tissues showed pentachlorophenol levels of 11.3 ppm in brain, 45.6 ppm in liver, and 20 ppm in kidney. Similar birds intentionally shot a few days later showed residues of less than 1 ppm in the same tissues; this is circumstantial evidence that birds also eliminate pentachlorophenol rapidly. Hill et al. (1975) reported dietary lethal concentrations (LC_{50}) for pentachlorophenol to be 3400 to 5200 ppm for bobwhite, Japanese quail, pheasant, and mallard duck.

Another economic problem is caused by the development of a musty taint in muscle and eggs of chickens. This problem apparently results when treated wood shavings are used for bedding. Fungi in the litter methylate tetrachlorophenols and pentachlorophenols to the corresponding anisoles (Curtis et al., 1972, 1974).

E.4.4.3 Effects on Mammals

E.4.4.3.1 General Considerations - Pentachlorophenol can be absorbed dermally, orally, or by inhalation. The acute lethal dose for all species tested is 100 to 200 mg/kg. The solvent used can affect the toxicity. The lethal multiple dose is 50 to 70 mg/kg. The toxicity of inhaled pentachlorophenol has not been adequately studied, and research should be done on this route of uptake.

Levels of 125 to 250 ppm pentachlorophenol in food cause adversion to the food by cats. In rats levels of 300 to 600 ppm result in decreased food consumption; levels of 30 to 60 ppm are eaten with little effect on food intake. Peak blood levels are reached 3 to 12 hr after ingestion. Most of the pentachlorophenol in blood is found in the plasma, with only small amounts in red blood cells. Pentachlorophenol is primarily excreted in the urine with a half-life of 1.5 to 4 days. The metabolites formed vary with different species. Evidence suggests that small amounts of pentachlorophenol may bind to plasma proteins and that enterohepatic circulation may prolong elimination. The mechanism of action involves the uncoupling of oxidative phosphorylation.

E.4.4.3.2 Effects on Domestic Animals - Domestic animals may be exposed to pentachlorophenol through gross negligence of the livestock owner who allows animals, especially cattle, to drink pentachlorophenol solutions or by casual contact with pentachlorophenol-treated wood in fences, feed bunks, bunker silos, and farm buildings. The direct consumption of pentachlorophenol solutions has caused death in animals; cattle are the species most likely to consume the solution. The half-life of pentachlorophenol in the blood of cattle is less than 2 days; surviving animals should not be slaughtered for 30 days after an acute exposure.

One important factor affecting the toxicity of pentachlorophenol in domestic animals is the carrier solvent used in treating wood. Apparently, a variety of hydrocarbons with high solubility for pentachlorophenol are used as cosolvents. From a toxicologic viewpoint, the types of oils and organic solvents used are not adequately specified; thus, it is not possible to determine the contribution of the solvent to the toxicity of pentachlorophenol. This is an area that needs study, particularly concerning carrier solvents used to treat wood that has a high probability of coming into significant contact with humans or animals.

With the exception of acute accidental exposures, the most serious incidents of documented pentachlorophenol toxicity to animals resulted from placing sows in farrowing crates or individual farrowing houses constructed of freshly treated lumber. Such practices have resulted in sow and piglet deaths. Using weathered lumber or adequate bedding prevents the problem. There did not appear to be any serious long-term health effects in surviving animals placed in clean environments.

More recently, it has been recognized that cattle may be exposed to low levels (<1 mg/kg) of pentachlorophenol by eating out of feed bunks constructed of treated wood, licking treated wood, or eating silage or other feed stored immediately adjacent to treated wood. The greater the tendency of the wood to bleed (i.e., the oil solution oozes to the surface), the higher will be the exposure. More attention to the type of wood treatment and uses of treated wood can prevent these low-level exposures. Dermal exposure is possible when cattle rub against treated boards or poles, but this route of exposure has not been documented nor quantitated. Such exposure would likely be minimal. Pentachlorophenol levels have not been measured in the air of animal housing containing pentachlorophenol-treated lumber. Consequently, respiratory exposure has not been quantitated. Studies monitoring these levels should be conducted. Calculations based on vapor densities show that the respiratory exposures should be low.

The acute lethal toxicity of pentachlorophenol in swine, sheep, and cattle is on the order of 140 to 160 mg/kg. Multiple doses at 35 to 70 mg/kg cause weight loss or decreased weight gain. Recovery occurs after termination of exposure. Long-term feeding studies in domestic animals at low levels of exposure have not been reported; studies are warranted in this area.

Acute dermal effects consisting of inflammation and necrosis have been observed in swine lying directly on freshly treated wood. Lesions observed at necropsy in acutely poisoned animals include renal edema and hemorrhage, generalized congestion of viscera, and inflammation in the digestive tract. Death occurs rapidly following a period of collapse and rapid breathing. Rigor mortis occurs in minutes instead of hours.

E.4.4.3.3 Effects on Laboratory Animals — The acute toxicity of pentachlorophenol to laboratory animals has been determined. In general, the oral LD_{50} values for rats, rabbits, and guinea pigs range from 70 to 300 mg/kg. The oral LD_{50} for aqueous solutions of sodium pentachlorophenate is 200 mg/kg; oil solutions of pentachlorophenol have an approximate LD_{50} of 140 mg/kg. The type of oil used affects the toxicity; the lowest LD_{50} (27 mg/kg) was from oral administration of a fuel oil solution containing 0.5% pentachlorophenol. The dermal lethal dose ranges from 40 to 200 mg/kg for pentachlorophenol and from 100 to 300 mg for sodium pentachlorophenate. The acute and chronic toxicities of inhaled pentachlorophenol have not been adequately reported; this area is an important void in the toxicologic information requiring immediate attention.

Chronic oral toxicity studies with rats have been reported by several investigators. One of the issues addressed in these studies has been the determination of the contribution of nonphenolic contaminants to the toxicity of pentachlorophenol. Pentachlorophenol is available commercially as technical pentachlorophenol containing 9 to 27 ppm hexachlorodibenzo-*p*-dioxin, 90 to 135 ppm heptachlorodibenzo-*p*-dioxin, and 500 to 2500 ppm octachlorodibenzo-*p*-dioxin or as technical pentachlorophenol with a low nonphenolic content (containing less than 60 ppm total dioxins). These studies have helped to identify no-effect levels as well as to define toxic levels and effects that aid in determining the toxicologic hazard associated with pentachlorophenol exposure.

Rats can tolerate rather remarkably high doses of pentachlorophenol for extended periods. Kimbrough and Linder (1975) fed rats technical and pure pentachlorophenol at levels of 1000 ppm (50 mg/kg) for 90 days. This dose is about one-third to one-fourth of the typical single-dose LD_{50} value. Rats fed pure pentachlorophenol showed some liver cell enlargement. The group fed technical pentachlorophenol had more liver cell changes, including foamy cytoplasm, single hepatocellular necrosis, and slight interstitial fibrosis.

Several adequately designed 90-day chronic toxicity studies have been reported. Knudsen et al. (1974) fed male and female rats technical pentachlorophenol in the diet at levels of 25, 50, and 200 ppm, which are equivalent to approximate dosage rates of 1.5, 3, and 14 mg/kg respectively. The authors considered 25 ppm to be the no-effect level. Liver enzyme induction occurred at 200 ppm. Hemoglobin and erythrocyte counts were depressed at 50 ppm in male rats, but no hematologic effects were observed at any dose in female rats. Liver pathology was limited to centilobular vacuolization, which was also seen to a lesser extent in controls.

Goldstein et al. (1977) demonstrated in an eight-month feeding study with rats that technical pentachlorophenol induced liver aryl hydrocarbon hydroxylase and glucuronyl transferase at feeding levels of 20 ppm (about 1.2 mg/kg) or higher. Pure pentachlorophenol was not a potent enzyme inducer; only the 500 ppm level increased glucuronyl transferase. Although biologic effects were observed, feeding levels of 500 ppm (about 30 mg/kg) for eight months was not particularly debilitating.

Kociba et al. (1971) compared the toxicities of technical pentachlorophenol and a 95/5 mixture of purified pentachlorophenol and tetrachlorophenol. They found a decrease in hemoglobin, erythrocyte count, and packed cell volume in male rats fed 30 mg technical pentachlorophenol per kilogram for 90 days. The purified 95/5 mixture had less biological effect than technical pentachlorophenol. For the 95/5 compound, 3 mg/kg produced no effect, and 10 mg/kg caused only a relative increase in liver weight. For technical pentachlorophenol, 3 mg/kg produced a small increase in serum alkaline phosphatase which was not apparent at 10 mg/kg. Knudsen et al. (1974) also found an increase in alkaline phosphatase at 1.5 and 14 mg/kg but not at 3 mg/kg. In the Kociba et al. (1971) study, 3 mg technical pentachlorophenol per kilogram caused a relative increase in liver and kidney weights; 10 mg/kg increased absolute liver weight.

Kociba et al. (1973) fed male and female rats pentachlorophenol with low nonphenolic content for 90 days. The no-effect level was 10 mg/kg in females and 3 mg/kg in males. The effects produced at 30 mg/kg were limited to changes in liver and kidney weights. Changes in liver and kidney weights are a biological response to large amounts of a toxicant that must be metabolized by the liver and excreted by the kidney. In all of the above studies the histopathologic lesions were minimal.

Schwetz et al. (1976, 1978) fed male and female rats pentachlorophenol with a low nonphenolic content for 22 or 24 months respectively. The no-effect level was 3 mg/kg in females and 10 mg/kg in males. As with the other studies cited, feeding 30 mg/kg for 22 or 24 months did not result in any life-impairing effects. The authors concluded that pentachlorophenol was not carcinogenic.

Pentachlorophenol is a commercial poison used because of its long-term stability as a wood preservative; therefore, it is fortunate that even high exposure rates for long periods do not result in serious health effects. Although some changes in clinical chemistry and hematologic parameters have been shown, the magnitude of the changes was small. The nonphenolic content of technical pentachlorophenol increases its biologi activity in rats primarily through liver enzyme induction and increases in liver weight. Based on current data, the inadvertant exposure of animals or humans to microgram per kilogram levels of pentachlorophenol is not expected to result in any detectable adverse toxicologic effects.

E.4.5 EFFECTS ON HUMANS

E.4.5.1 Exposure Factors

E.4.5.1.1 General Population — The literature indicates that at least some segments of the general population are exposed to low levels of pentachlorophenol. Pentachlorophenol has been found in 1% to 3% of food composites in market basket surveys; estimated daily exposures ranged from 1 to 6 μg per person for the years 1965 to 1969 (Duggan and Corneliussen, 1972). Pentachlorophenol was found in drinking water at 60 ppt in Oregon (Buhler, Rasmusson, and Nakaue, 1973). The origin of the pentachlorophenol is unclear; some evidence shows that chlorination of water will result in the chlorination of naturally occurring phenols (Arsenault, 1976). Pentachlorophenol was also found in sewage influent and effluent (Buhler, Rasmusson, and Nakaue, 1973).

Casarett et al. (1969) reported pentachlorophenol in plasma (0.05 to 1 ppm) and urine (0.01 to 10 ppm) of nonoccupationally exposed persons in Hawaii. Shafik (1973) found an average of 26.3 ppb pentachlorophenol in 18 human fat samples of unspecified origin. Cranmer and Freal (1970) reported levels of 2 to 11 ppb in urine from the general population. No adverse health effects have been ascribed to these exposure levels. The findings of Braun, Blau, and Chenoweth (1978) showing that a 0.1 mg/kg dose in humans results in a steady-state plasma level of 0.49 ppm, the results of laboratory animal studies, and the minimal effects reported in higher occupational exposures suggest that present levels of exposure will not result in adverse health effects. Additionally, it is not known to what extent the present pentachlorophenol residues in humans result from use of pentachlorophenol as a commercial poison, from water chlorination, or from degradation of compounds such as lindane or hexachlorobenzene.

E.4.5.1.2 Industrial Workers -- Four groups of industrial workers have varying degrees of exposure to pentachlorophenol. The first is the rather small group of workers employed in the three or four chemical plants actually manufacturing pentachlorophenol. Industrial hygiene programs are operational in these plants. Summary reports have not indicated any serious health problems, although dermal sensitivity was noted in some instances. Exposure factors have not been reported. The trend in the industry has been toward reducing exposure by use of bulk handling systems and formulation of large solid pentachlorophenol blocks. Insufficient information is available in the open literature to fully assess this situation.

The second group includes workers involved in formulating and packaging pentachlorophenol for particular uses. No information was found regarding the extent of this type of industrial exposure.

The third group consists of workers in several hundred wood treating plants using pentachlorophenol. Some wood treatment plants are operated by large organizations with an awareness of good industrial hygiene. Other wood treating facilities are small, low-volume companies, and there is very little information on their hygiene practices and worker exposure. Pentachlorophenol has been identified in the air in wood treating plants. Levels of pentachlorophenol in air have ranged from 0.006 to 0.297 mg/m^3 (Wyllie et al., 1975; Arsenault, 1976). The highest value represents the maximum level that occurs when a pressure treating cylinder is opened. Typical air levels appear to be 0.01 to 0.02 mg/m^3. Because of limited data it is not known if these values are representative of the industry or are unique to one situation.

Workers in two wood treating plants in Hawaii have been studied for several years. Plasma levels of 1 to 20 ppm pentachlorophenol and urinary levels of 1 to 20 ppm were found. Levels decreased rapidly when work exposure was stopped for several days. A variety of medical tests showed no significant adverse health effects following chronic exposures. Some evidence of higher levels of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvate transaminase, and lactic dehydrogenase enzymes were reported (Klemmer, 1972); however, the values were not outside normal ranges. A more detailed statistical analysis is in progress. Takahashi et al. (1975) reported elevated levels of C-reactive protein in plasma of chronically exposed workers. The relationship of this occurrence to dermal or respiratory inflammation is unknown. There was increased incidence of skin inflammation in the group exposed to pentachlorophenol. Some evidence suggests reversible effects on kidney function consisting of decreased creatinine clearance and phosphorus reabsorption.

The fourth group of workers at occupational risk are builders using large amounts of treated wood. Skin irritation has occurred. Verbal reports of irritating vapors have been made, but apparently the problem has not been sufficient to result in a careful clinical case study, documentation, and reporting. The role of solvent vapors in these situations is also a factor that has not received attention. The use of protective clothing and adequate ventilation apparently prevent or resolve most of these problems.

E.4.5.2 Toxicity

Humans are potentially exposed to pentachlorophenol via dermal, respiratory, and oral routes, and fatal pentachlorophenol toxicosis has occurred. According to the literature, lethal exposures have resulted from extensive dermal contact with pentachlorophenol solutions, intentional ingestion, gross contamination of a container used to prepare food, and, in several cases, inhalation of dust. As with other commercial poisons, failure to observe good hygiene practices, to use common sense, and to utilize protective clothing leads to excessive exposures that can result in toxicosis.

There are instances recorded in which pentachlorophenol solutions have been used in large quantities to treat wood surfaces inside of houses. Often these have involved treating woods such as cedar and redwood which may not take up and hold the solution as well as other woods. Adverse health effects have occurred as a result of breathing either vapors or minute particles that result from pentachlorophenol blooming, which occasionally occurs.

Pentachlorophenol is irritating to respiratory membranes and to skin. Dermal exposures often involve contact with petroleum-based solvents; thus, the contribution of pentachlorophenol versus the contribution of the solvent to the resulting toxicologic response has not been determined. Air concentrations greater than 1 mg/m^3 cause painful irritation in the upper respiratory tract and result in sneezing and coughing, particularly in newly exposed individuals. Concentrations as high as 2.4 mg/m^3 can be tolerated by those conditioned to exposures.

Symptoms of both acute exposures and chronic high-level exposures include general weakness, fatigue, dizziness, headache, anorexia, nausea and vomiting, dyspnea, hyperpyrexia, respiratory distress, tachycardia, and profuse perspiration. In addition, chronic poisoning may include weight loss and hepatomegaly. Treatment of intoxications should include administration of balanced intravenous fluids to promote renal excretion, with close attention paid to acid-base balance and electrolytes (Haley, 1977). Exchange blood transfusions have been used in intoxicated babies with apparent success (Robson et al., 1969).

Symptoms occur at blood levels of 40 to 80 ppm. Acute intoxications have resulted in blood and tissue levels of 100 to 200 ppm. An oral dose of 0.1 mg/kg resulted in a peak plasma level of 0.248 ppm, with a calculated steady-state value of 0.491 ppm after eight days. Pentachlorophenol is primarily eliminated in the urine. Most of the chemical in the urine is unchanged pentachlorophenol, with lesser amounts of pentachlorophenol glucuronide. Small amounts of absorbed pentachlorophenol are eliminated in the feces as pentachlorophenol and pentachlorophenol glucuronide. The half-life for elimination is approximately 30 hr. There is some speculation of a longer half-life for a low pentachlorophenol residue. Presently reported studies are inadequate to clarify this point for one or more of the following reasons: (1) continuous low-level exposure could have occurred, (2) the length of the study was too short, or (3) the levels of exposure were low. If there is a long-term retention of small amounts of pentachlorophenol, it may result from specific high-affinity protein binding or enterohepatic circulation. The available data indicate that pentachlorophenol does not have a long-term accumulation and storage in body fat as do some other chlorinated hydrocarbons.

E.4.6 TERATOGENIC AND CARCINOGENIC EFFECTS

Pentachlorophenol has not shown mutagenic activity in the Ames test (Anderson, Leighty, and Takahashi, 1972), the host-mediated assay (Buselmaier, Röhrborn, and Propping, 1973), or the sex-linked lethal test on drosophila (Vogel and Chandler, 1974). Schwetz et al. (1978) reported that pentachlorophenol was not carcinogenic when fed to rats for 22 or 24 months. Innes et al. (1969) reported that technical pentachlorophenol at a dietary level of 130 ppm was not tumorigenic in rats. Schwetz, Keeler, and Gehring (1974) found both purified and technical pentachlorophenol to be embryotoxic and fetotoxic but not teratogenic in rats. The no-effect level was 5 mg/kg.

E.4.7 STANDARDS AND REGULATIONS

The maximum concentration in air established by the American Industrial Hygiene Association (1970) is 0.5 mg pentachlorophenol or 0.5 mg sodium pentachlorophenate per cubic meter. There is a threshold limit value for an 8-hr exposure. The Code of Federal Regulations 21, part 121, paragraph 121:2556, allows up to 50 ppm pentachlorophenol in treated wood intended for use in contact with food. A tolerance for pentachlorophenol in food has not been established.

E.4.8 RECOMMENDATIONS

1. More information on accepted and safe uses of pentachlorophenol-treated wood should be made available to the end user. Presently, anyone can purchase treated wood at a lumber yard without being cautioned to avoid skin contact or receiving information on herbicidal properties.

2. Research on the biologic fate of pentachlorophenol in fish and mammals is needed to determine if long-term cellular or plasma-protein binding occurs.

3. The toxicity of the solvents used to carry pentachlorophenol into wood should be assessed.

4. Ambient pentachlorophenol levels in air of closed livestock facilities need to be determined in order to fully assess the potential hazard.

5. The sources of pentachlorophenol residues in food products should be determined.

PART E

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16. ABSTRACT

This report is a review of the scientific literature on the biological and environmental effects of chlorophenols. Included in the review are a general summary and a comprehensive discussion of the following topics as related to chlorophenols and specific chlorophenol compounds: physical and chemical properties; occurrence; synthesis and use; analytical methodology; biological aspects in microorganisms, plants, wild and domestic animals, and humans; distribution, mobility, and persistence in the environment; assessment of present and potential health and environmental hazards; and review of standards and governmental regulations.

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